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<p>(54) Title: DNA SEQUENCES AND PLASMIDS FOR THE PREPARATION OF PLANTS WITH CHANGED SUCROSE CONCENTRATION</p> <p>(57) Abstract</p> <p>DNA sequences are described, that by integration in a plant genome cause the activity of the sucrose-phosphate-synthase (SPS) of the plant to be changed, plasmids, containing these DNA sequences as well as transgenic plants that by introduction of the DNA sequences causes changes in the activity of sucrose-phosphate-synthase.</p>		

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Title: DNA sequences and plasmids for the preparation of plants with changed sucrose concentration

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Field of the invention

The present invention relates to DNA sequences and plasmids, containing these DNA sequences, which by integration into a plant genome, cause the activity of the sucrose-phosphate-synthase (SPS) of the plant to be changed and thus affect the sugar metabolism of the plant. The invention further relates to transgenic plants, in which through introduction of the DNA sequences, changes in the activity of the sucrose-phosphate-synthase are produced.

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Sucrose is of central importance for the plant and serves many functions. For the long distance transport of photoassimilates and/or energy between various organs in plants, sucrose is almost exclusively used. The sucrose, which is transported in a specific heterotrophic organ, determines the growth and the development of this organ. Thus it is known, e.g. from EP 442 592, that transgenic plants, in which the transport away of the sucrose from the exporting leaves is inhibited by expression of an apoplastic invertase, shows a strong reduction in the growth of e.g. roots or tubers in the case of potato plants. For tobacco plants, the principal importance of sucrose as the central function for the long distance transport of energy carriers within the plant is described (von Schaewen et al, 1990, EMBO J 9: 3033-3044).

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Whilst it has been clearly shown that a reduction of the amount of sucrose imported in the heterotrophic organs, such as tubers and seeds, leads to loss of yield, it is

not known whether an increase in the amount of sucrose in the photosynthetically active parts of the plant, mainly the leaves, leads to a better supply of heterotrophic organs and thus to an increase in yield.

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A second central role for sucrose and/or the hexoses, glucose and fructose, derived from sucrose, is in the protection of plants against frost damage at low temperatures. Frost damage is one of the main limiting factors in agricultural productivity in the northern hemisphere. Temperatures below freezing lead to the formation of ice crystals. Since the growing ice crystals consist of pure water, water is abstracted from the cells as the temperature falls.

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This dehydration has at least two potential damaging results:

- a) all dissolved substances within a cell are strongly concentrated and the cell contracts following the loss of water. Highly concentrated salts and organic acids lead to membrane damage;
- b) with rehydration from dew, the previously contacted cells reexpand. The cell membrane also expands again. The volume expansion puts a heavy mechanical load on the membrane.

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It is thus clear that a freezing/dew cycle can lead to severe membrane damage of the cells and thus to damage to the plant.

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It thus appears worth trying to hinder the freezing. One possible strategy is the increased formation of osmotically active substances in the cytosol of plant cells. This should lead to a lowering of the freezing point. Osmotically active substances include sucrose

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and/or the two hexoses derived from sucrose.

The increased formation of sucrose and/or the two hexoses at low temperatures is desirable in the growing plant.

5 Another situation can exist in the harvested parts of a plant, especially in storage. For example, in potato tubers that are stored at 4-8°C, hexoses (glucose) accumulate. It would appear to be sensible, to see this as the answer to a lowering of the temperature
10 ("cold-sweetening").

The accumulation of sucrose and glucose has in the case of potato tubers economically undesirable results. Increased amounts of reducing sugars, such as glucose, in potatoes
15 which are fried when preparing crisps, chips and the like, leads to an undesirable browning due to the Maillard reaction. Such products with a dark brown colour are not generally acceptable to the consumer. Further the cooking strength is strongly dependent on the content of starch
20 and/or its breakdown products which are important in determining the quality characteristics of the potato.

In relation to the economic aspects, sucrose thus possesses three especially important functions:

- 25 1 as the transport form for the distant transport of photoassimilates,
2 as an osmotically active substance with the desirable activity of lowering the freezing point in intact, growing plants, and
30 3 in the undesirable formation of reducing sugars in stored harvested parts of a plant, e.g. the potato tubers, as a result of low temperatures.

The biosynthesis pathways for the formation of sucrose,
35 either from the primary photosynthesis products (in the

leaf) or by breakdown of starch (in the storage organs e.g. of potatoes), are known. An enzyme in sucrose metabolism is sucrose-phosphate-synthase (SPS). It forms sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate, which in a second step is converted to sucrose.

The isolation of SPS from maize and the cloning of a cDNA from mRNA from maize tissue is known (EP 466 995). In this application, processes for the purification of a protein such as by centrifuging of homogenates, differential precipitation and chromatography are described. A 300 times enrichment of SPS from plant tissue has been described by Salerno and Pontis (Planta 142: 41-48, 1978).

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In view of the significance of SPS for carbohydrate metabolism it is questionable whether plants tolerate a reduction in SPS activity in all or in certain organs. It is especially not known, whether it is possible to produce transgenic plants with a reduced SPS activity. Also the use of SPS for the modification of the functions of sucrose for lowering the freezing point in intact plants and for the formation of reducing sugars in harvested parts is not known.

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For the preparation of plants with reduced SPS activity, i.e. plants with changed sucrose concentration, it is necessary to make available an SPS coding region of such plant species, for which processes are described, whereby transgenic plants can be grown in large numbers. In as much as a reduction of SPS activity can be achieved, by selection from a large amount, the possibility exists of obtaining plants with such a phenotype. Further organ specific promoters for gene expression should exist for

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the plant species, by which the possibility of an organ specific reduction of the SPS activity could be investigated.

- 5 A species which fulfils the stated requirements is *Solanum tuberosum*. The genetic modification of *Solanum tuberosum* by gene transfer using *Agrobacteria* is well described (Fraley et al., 1985, Crit Rev Plant Sci 4: 1-46). Promoters for leaf specific (Stockhaus et al., 1989, Plant
10 Cell 1: 805-813), tuber specific (EP 375 092) and wound inducing (EP 375 091) gene expression are known.

The present invention now provides DNA sequences with which changes of SPS activity are actually and
15 demonstrably possible and with which the sucrose concentration in the plant can be modified. It is concerned with sequences with the coding region of sucrose-phosphate-synthase (SPS) from *Solanum tuberosum*.

20 These DNA sequences can be introduced in plasmids and thereby combined with steering elements for expression in eukaryotic cells. Such steering elements are on the one hand transcription promoters and on the other hand transcription terminators.

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Each plasmid comprises:

- a) a suitable promoter, that ensures that the coding sequence is read off at the suitable time point and/or in a specified development stage in the
30 transgenic plants or in specified tissues of transgenic plants,
- b) at least one coding sequence, that
- i) is so coupled to the promoter that the formation of translatable RNA is allowed in a
35 protein, whereby the protein demonstrates

enzymatic activity, that leads to a modification of the sucrose concentration in the plant, or

- 5 ii) is so coupled to the promoter that the non-coding strand is read off, which leads to the formation of a so-called "anti-sense" RNA, which suppresses the formation of the coding protein of an endogenous gene in the plant, which is involved in the sucrose biosynthesis, and
- 10

- c) a non-coding termination sequence, that contains the signals for the termination and polyadenylation of the transcript.

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The present invention further provides plasmids in which there are the DNA sequences which change the SPS activity in the plant.

- 20 The coding sequences named under b) are the SPS sequences with the following nucleotide sequences:

SPS 1 sequence (Seq. ID No.1):

CTATTCTCTC CCCTCCTTTT TCTCCTCTCT TCAACCCCAA AACTTCCCTT TCAAAGCCTT 60
TGCTTTCCCT TTCTCACTTA CCCAGATCAA CTAAGCCAAT TTGCTGTAGC CTCAGAAAAC 120
AGCATTCCCA GATTGAAAAA GAATCTTTTT CAGTACCCAA AAGTTGGGTT TCTCATGTCC 180
AGCAAGGATT AGCTGCTCTA GCTATTTCTT TAGCCCTTAA TTTTGTCCA GTTGTGTCTT 240
CTGATTCTGC ATTGGCATCT GAATTTGATG TGTTAAATGA AGGGCCACCA AAGGACTCAT 300
ATGTAGTTGA TGATGCTGGT GTGCTTAGCA GGGTGACAAA GTCTGATTTG AAGGCATTGT 360

TGTCTGATGT GGAGAAGAGA AAAGGCTTCC ACATTAATTT CATCACTGTC CGCAAGCTCA 420
 CTAGCAAAGC TGATGCTTTT GAGTATGCTG ACCAAGTTTT GGAGAAGTGG TACCCTAGTG 480
 TTGAACAAGG AAATGATAAG GGTATAGTTG TGCTTGTTAC AAGTCAAAAG GAAGGCGCAA 540
 TAACCGGTGG CCCTGATTTT GTAAAGGCCG TTGGAGATAC TGTTCTTGAT GCTACCGTCT 600
 CAGAGAACCT TCCAGTGTG GCTACTGAAG AGAAGTACAA TGAAGCAGTT TTCAGCACTG 660
 CCACACGTCT TGTTGCAGCC ATTGATGGCC TTCCTGATCC TGGTGGACCC CAACTCAAGG 720
 ATAACAAAAG AGAGTCCAAC TTCAAATCCA GAGAGGAAAC TGATGAGAAA AGAGGACAAT 780
 TCACACTTGT GGTGGTGGG CTGTTAGTGA TTGCTTTTGT TGTTCCCTATG GCTCAATACT 840
 ATGCATATGT TTCAAAGAAG TGAAGTGTCT GATTCTGGAA AGTTACATTT TCGTGAGATT 900
 TGAGTAAGCA TGTATATTAT CGTGTAACAA ATGGTCCATT CGGAAATGAC TGATTG 956
 ATG AGA TAT TTA AAA AGG ATA AAT ATG AAG ATT TGG ACC TCC CCT 1001
 Met Arg Tyr Leu Lys Arg Ile Asn Met Lys Ile Trp Thr Ser Pro
 1 5 10 15
 AAC ATA ACG GAT ACT GCC ATT TCT TTT TCA GAG ATG CTG ACG CCA 1046
 Asn Ile Thr Asp Thr Ala Ile Ser Phe Ser Glu Met Leu Thr Pro
 20 25 30
 ATA AGT ACA GAC GGC TTG ATG ACT GAG ATG GGG GAG AGT AGT GGT 1091
 Ile Ser Thr Asp Gly Leu Met Thr Glu Met Gly Glu Ser Ser Gly
 35 40 45

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GCT TAT ATT ATT CGC ATT CCT TTT GGA CCA AGA GAG AAA TAT ATT	1136
Ala Tyr Ile Ile Arg Ile Pro Phe Gly Pro Arg Glu Lys Tyr Ile	
50 55 60	
CCA AAA GAA CAG CTA TGG CCC TAT ATT CCC GAA TTT GTT GAT GGT	1181
Pro Lys Glu Gln Leu Trp Pro Tyr Ile Pro Glu Phe Val Asp Gly	
65 70 75	
GCA CTT AAC CAT ATT ATT CAA ATG TCC AAA GTT CTT GGG GAG CAA	1226
Ala Leu Asn His Ile Ile Gln Met Ser Lys Val Leu Gly Glu Gln	
80 85 90	
ATT GGT AGT GGC TAT CCT GTG TGG CCT GTT GCC ATA CAC GGA CAT	1271
Ile Gly Ser Gly Tyr Pro Val Trp Pro Val Ala Ile His Gly His	
95 100 105	
TAT GCT GAT GCT GGC GAC TCA GCT GCT CTC CTG TCA GGT GCT TTA	1316
Tyr Ala Asp Ala Gly Asp Ser Ala Ala Leu Leu Ser Gly Ala Leu	
110 115 120	
AAT GTA CCA ATG CTT TTC ACT GGT CAC TCA CTT GGT AGA GAT AAG	1361
Asn Val Pro Met Leu Phe Thr Gly His Ser Leu Gly Arg Asp Lys	
125 130 135	
TTG GAG CAA CTG TTG CGA CAA GGT CGT TTG TCA AAG GAT GAA ATA	1406
Leu Glu Gln Leu Leu Arg Gln Gly Arg Leu Ser Lys Asp Glu Ile	
140 145 150	
AAC TCA ACC TAC AAG ATA ATG CGG AGA ATA GAG GCT GAA GAA TTA	1451
Asn Ser Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu Glu Leu	
155 160 165	

ACT CTT GAT GCT TCC GAA ATT GTC ATC ACT AGT ACA AGA CAG GAG	1496
Thr Leu Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg Gln Glu	
170 175 180	
ATT GAC GAG CAA TGG CGT TTG TAT GAT GGG TTT GAT CCA ATA TTA	1541
Ile Asp Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro Ile Leu	
185 190 195	
GAG CGT AAG TTA CGT GCA AGG ATC AAG CGC AAT GTC AGC TGT TAT	1586
Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser Cys Tyr	
200 205 210	
GGC AGG TTT ATG CCT CGT ATG GCT GTA ATT CCT CCT GGG ATG GAG	1631
Gly Arg Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly Met Glu	
215 220 225	
TTC CAC CAT ATT GTG CCA CAT GAA GGT GAC ATG GAT GGA GAA ACA	1676
Phe His His Ile Val Pro His Glu Gly Asp Met Asp Gly Glu Thr	
230 235 240	
GAA GGA AGT GAA GAT GGG AAG ACC CCG GAT CCA CCT ATT TGG GCA	1721
Glu Gly Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile Trp Ala	
245 250 255	
GAG ATT ATG CGC TTC TTT TCT AAT CCA AGG AAG CCT ATG ATA CTC	1766
Glu Ile Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met Ile Leu	
260 265 270	
GCA CTT GCT AGG CCT GAT CCC AAG AAG AAC CTC ACT ACT TTA GTG	1811
Ala Leu Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr Leu Val	
275 280 285	
AAA GCA TTT GGT GAA TGT CGT CCA TTG AGA GAG CTT GCT AAT CTT	1856
Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu	
290 295 300	

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ACT TTG ATA ATG GGT AAT CGA GAT AAT ATC GAC GAA ATG TCT AGC	1901
Thr Leu Ile Met Gly Asn Arg Asp Asn Ile Asp Glu Met Ser Ser	
305 310 315	
ACC AAT TCT GCA CTT CTT CTT TCA ATC TTG AAA ATG ATA GAT AAG	1946
Thr Asn Ser Ala Leu Leu Leu Ser Ile Leu Lys Met Ile Asp Lys	
320 325 330	
TAT GAT CTT TAT GGT CAA GTA GCT TAT CCT AAA CAC CAC AAG CAG	1991
Tyr Asp Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys Gln	
335 340 345	
TCA GAT GTT CCT GAT ATC TAC CGT CTT GCT GCA AAG ACT AAG GGT	2036
Ser Asp Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly	
350 355 360	
GTT TTT ATT AAT CCA GCT TTT ATT GAG CCT TTT GGA CTG ACT TTG	2081
Val Phe Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu Thr Leu	
365 370 375	
ATT GAG GCA GCA GCT TAT GGT CTC CCA ATG GTA GCC ACA AAA AAT	2126
Ile Glu Ala Ala Ala Tyr Gly Leu Pro Met Val Ala Thr Lys Asn	
380 385 390	
GGA GGA CCT GTT GAT ATA CAT AGG GTT CTT GAC AAT GGT CTC TTA	2171
Gly Gly Pro Val Asp Ile His Arg Val Leu Asp Asn Gly Leu Leu	
395 400 405	
GTG GAT CCC CAT GAT CAG CAG GCA ATT GCT GAT GCT CTT TTG AAG	2216
Val Asp Pro His Asp Gln Gln Ala Ile Ala Asp Ala Leu Leu Lys	
410 415 420	
TTG GTT GCT GAT AAG CAA CTG TGG GCT AAA TGC AGG GCA AAT GGA	2261
Leu Val Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala Asn Gly	
425 430 435	

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TTA AAA AAT ATC CAC CTT TTC TCA TGG CCC GAG CAC TGT AAA ACT	2306
Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His Cys Lys Thr	
440 445 450	
TAT CTA TCC CGG ATA GCT AGC TGC AAA CCA AGG CAA CCA CGC TGG	2351
Tyr Leu Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln Pro Arg Trp	
455 460 465	
CTG AGA TCC ATT GAT GAT GAT GAT GAA AAT TCA GAA ACA GAT TCA	2396
Leu Arg Ser Ile Asp Asp Asp Asp Glu Asn Ser Glu Thr Asp Ser	
470 475 480	
CCT AGT GAT TCC TTG AGA GAT ATT CAT GAT ATA TCT CTG AAT TTG	2441
Pro Ser Asp Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu	
485 490 495	
AGA TTT TCA TTA GAT GGG GAA AAG AAT GAC AAT AAA GAA AAT GCT	2486
Arg Phe Ser Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala	
500 505 510	
GAT AAT ACA TTA GAC CCC GAA GTT CGA AGG AGC AAG TTA GAG AAT	2531
Asp Asn Thr Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn	
515 520 525	
GCT GTT TTG TCC TTA TCT AAG GGT GCA CTG AAG AGC ACA TCA AAA	2576
Ala Val Leu Ser Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys	
530 535 540	
TCT TGG TCG TCA GAC AAG GCA GAC CAA AAC CCT GGT GCT GGT AAA	2621
Ser Trp Ser Ser Asp Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys	
545 550 555	
TTC CCA GCG ATT AGG AGG AGG CGA CAT ATT TTT GTT ATT GCA GTG	2666
Phe Pro Ala Ile Arg Arg Arg Arg His Ile Phe Val Ile Ala Val	
560 560 565	

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12

GAT TGT GAT GCT AGC TCA GGA CTC TCT GGA AGT GTG AAA AAG ATA	2711
Asp Cys Asp Ala Ser Ser Gly Leu Ser Gly Ser Val Lys Lys Ile	
570 575 580	
TTT GAG GCT GTA GAG AAG GAA AGG GCA GAG GGT TCC ATT GGA TTT	2756
Phe Glu Ala Val Glu Lys Glu Arg Ala Glu Gly Ser Ile Gly Phe	
585 590 595	
ATC CTG GCT ACA TCT TTC AAT ATA TCA GAA GTA CAG TCT TTC CTG	2801
Ile Leu Ala Thr Ser Phe Asn Ile Ser Glu Val Gln Ser Phe Leu	
600 605 610	
CTT TCA GAG GGC ATG AAT CCT ACT GAT TTT GAT GCT TAC ATA TGC	2846
Leu Ser Glu Gly Met Asn Pro Thr Asp Phe Asp Ala Tyr Ile Cys	
615 620 625	
AAT AGT GGT GGT GAT CTT TAT TAT TCG TCC TTC CAT TCT GAG CAA	2891
Asn Ser Gly Gly Asp Leu Tyr Tyr Ser Ser Phe His Ser Glu Gln	
630 635 640	
AAT CCT TTT GTA GTT GAC TTG TAC TAT CAC TCA CAT ATT GAG TAT	2936
Asn Pro Phe Val Val Asp Leu Tyr Tyr His Ser His Ile Glu Tyr	
645 650 655	
CGT TGG GGG GGC GAA GGA TTG AGA AAG ACT TTG GTG CGT TGG GCC	2981
Arg Trp Gly Gly Glu Gly Leu Arg Lys Thr Leu Val Arg Trp Ala	
660 665 670	
GCC TCT ATC ATT GAT AAG AAT GGT GAA AAT GGA GAT CAC ATT GTT	3026
Ala Ser Ile Ile Asp Lys Asn Gly Glu Asn Gly Asp His Ile Val	
675 680 685	
GTT GAG GAT GAA GAC AAT TCA GCT GAC TAC TGC TAT ACT TTC AAA	3071
Val Glu Asp Glu Asp Asn Ser Ala Asp Tyr Cys Tyr Thr Phe Lys	
690 695 700	

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GTC TGC AAG CCT GGG ACG GTT CCT CCA TCT AAA GAG CTT AGA AAA	3116
Val Cys Lys Pro Gly Thr Val Pro Pro Ser Lys Glu Leu Arg Lys	
705 710 715	
GTA ATG CGA ATT CAG GCA CTT CGT TGT CAC GCT GTT TAT TGT CAA	3161
Val Met Arg Ile Gln Ala Leu Arg Cys His Ala Val Tyr Cys Gln	
720 725 730	
AAT GGG AGT AGG ATT AAT GTG ATC CCT GTA CTG GCA TCT CGG TCC	3206
Asn Gly Ser Arg Ile Asn Val Ile Pro Val Leu Ala Ser Arg Ser	
735 740 745	
CAA GCA CTC AGG TAC TTA TAT CTG CGA TGG GGA ATG GAC TTG TCG	3251
Gln Ala Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp Leu Ser	
750 755 760	
AAG TTG GTG GTT TTC GTC GGA GAA AGT GGT GAT ACC GAT TAT GAA	3296
Lys Leu Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu	
765 770 775	
GGA TTA ATC GGT GGT CTA CGC AAG GCT GTC ATA ATG AAA GGC CTC	3341
Gly Leu Ile Gly Gly Leu Arg Lys Ala Val Ile Met Lys Gly Leu	
780 785 790	
TGC ACT AAT GCA AGC AGC TTA ATT CAC GGT AAT AGG AAT TAC CCG	3386
Cys Thr Asn Ala Ser Ser Leu Ile His Gly Asn Arg Asn Tyr Pro	
795 800 805	
CTA TCT GAT GTT TTA CCA TTC GAC AGC CCT AAT GTC ATC CAA GCG	3431
Leu Ser Asp Val Leu Pro Phe Asp Ser Pro Asn Val Ile Gln Ala	
810 815 820	
GAC GAG GAA TGT AGC AGC ACC GAA ATC CGT TGC TTA CTG GTG AAA	3476
Asp Glu Glu Cys Ser Ser Thr Glu Ile Arg Cys Leu Leu Val Lys	
825 830 835	

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CTA GCG GTA CTC AAA GGA TAATACCCTT CCCCCTTGA TTGTCAAAAA 3524

Leu Ala Val Leu Lys Gly

840

CCTATATGAG CTATAAGACT ATGCCATGAA AAGAATGGCC ATCCATTGG CTTGTCTTTT 3584

GAAGCTGTTA ATACTTTTCA ACAGACTACA AAATGAGATG AGTCCTTTGA TCCTCTTTAA 3644

AGGACATAAA AGCTTTATGC AAGAACCAGT GCTGTAAAGT TATAGAATTT CTTTGTCTAT 3704

ATATGACATT CGACAGAACC TGTTCCGGTT CATCGA 3740

SPS 2 sequence (Seq. ID No. 2)

ATTTTTTTCT CTAAGTTCTC TCTCGCTGTC CTTATCATTT CACCACCTCC ATAAATCTAG 60

AAACATCTTT TCTACTCCGT TAATCTCTCT AGCACACGGC GGAGGAGTGC GGCGGAGGAG 120

ATG GCG GGA AAC GAT TGG ATT AAC AGT TAC TTA GAG GCG ATA CTG 165

Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu

1 5 10 15

GAT GTT GGA CCA GGG CTA GAT GAT AAG AAG TCA TCG TTG TTG TTG 210

Asp Val Gly Pro Gly Leu Asp Asp Lys Lys Ser Ser Leu Leu Leu

20 25 30

AGA GAA AGA GGG AGG TTT AGT CCG ACG AGG TAC TTT GTT GAG GAA 255

Arg Glu Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu

35 40 45

GTT ATT ACT GGA TTC GAT GAG ACT GAT TTG CAT CGT TCG TGG ATC 300

Val Ile Thr Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Ile

50 55 60

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CGA GCA CAA GCT ACT CGG AGT CCG CAG AGA AGG AAT ACT AGG CTC	345
Arg Ala Gln Ala Thr Arg Ser Pro Gln Arg Arg Asn Thr Arg Leu	
65 70 75	
GAG AAT ATG TGC TGG AGG ATT TGG AAT TTG GCT CGC CAG AAA AAG	390
Glu Asn Met Cys Trp Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys	
80 85 90	
CAG CTT GAG GGA GAG CAA GCT CAG TGG ATG GCA AAA CGC CGT CAA	435
Gln Leu Glu Gly Glu Gln Ala Gln Trp Met Ala Lys Arg Arg Gln	
95 100 105	
GAA CGT GAA AGA GGT CGC AGA GAA GCA GTT GCT GAT ATG TCA GAG	480
Glu Arg Glu Arg Gly Arg Arg Glu Ala Val Ala Asp Met Ser Glu	
110 115 120	
GAT CTA TCT GAG GGA GAG AAA GGA GAT ATA GTC GCT GAC ATG TCA	525
Asp Leu Ser Glu Gly Glu Lys Gly Asp Ile Val Ala Asp Met Ser	
125 130 135	
TCT CAT GGT GAA AGT ACC AGA GGC CGA TTG CCT AGA ATC AGT TCT	570
Ser His Gly Glu Ser Thr Arg Gly Arg Leu Pro Arg Ile Ser Ser	
140 145 150	
GTT GAG ACA ATG GAA GCA TGG GTC AGT CAG CAG AGA GGA AAG AAG	615
Val Glu Thr Met Glu Ala Trp Val Ser Gln Gln Arg Gly Lys Lys	
155 160 165	
CTT TAT ATC GTG CTT ATA AGT TTA CAT GGT TTA ATT CGG GGT GAG	660
Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu Ile Arg Gly Glu	
170 -175 180	
AAT ATG GAG CTT GGA CGG GAT TCT GAT ACT GGT GGT CAG GTG AAG	705
Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys	
185 190 195	

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TAT GTT GTT GAA CTT GCG AGG GCC TTA GGG TCG ATG CCA GGT GTA	750
Tyr Val Val Glu Leu Ala Arg Ala Leu Gly Ser Met Pro Gly Val	
200 205 210	
 TAT CGG GTT GAC TTG CTT ACT AGA CAA GTA TCT TCA CCA GAA GTA	795
Tyr Arg Val Asp Leu Leu Thr Arg Gln Val Ser Ser Pro Glu Val	
215 220 225	
 GAT TGG AGC TAT GGT GAG CCG ACA GAG ATG CTG ACG CCA ATA AGT	840
Asp Trp Ser Tyr Gly Glu Pro Thr Glu Met Leu Thr Pro Ile Ser	
230 235 240	
 ACA GAC GGC TTG ATG ACT GAG ATG GGG GAG AGT AGT GGT GCT TAT	885
Thr Asp Gly Leu Met Thr Glu Met Gly Glu Ser Ser Gly Ala Tyr	
245 250 255	
 ATT ATT CGC ATT CCT TTT GGA CCA AGA GAG AAA TAT ATT CCA AAA	930
Ile Ile Arg Ile Pro Phe Gly Pro Arg Glu Lys Tyr Ile Pro Lys	
260 265 270	
 GAA CAG CTA TGG CCC TAT ATT CCC GAA TTT GTT GAT GGT GCA CTT	975
Glu Gln Leu Trp Pro Tyr Ile Pro Glu Phe Val Asp Gly Ala Leu	
275 280 285	
 AAC CAT ATT ATT CAA ATG TCC AAA GTT CTT GGG GAG CAA ATT GGT	1020
Asn His Ile Ile Gln Met Ser Lys Val Leu Gly Glu Gln Ile Gly	
290 295 300	
 AGT GGC TAT CCT GTG TGG CCT GTT GCC ATA CAC GGA CAT TAT GCT	1065
Ser Gly Tyr Pro Val Trp Pro Val Ala Ile His Gly His Tyr Ala	
305 310 315	
 GAT GCT GGC GAC TCA GCT GCT CTC CTG TCA GGT GCT TTA AAT GTA	1110
Asp Ala Gly Asp Ser Ala Ala Leu Leu Ser Gly Ala Leu Asn Val	
320 330 335	

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CCA ATG CTT TTC ACT GGT CAC TCA CTT GGT AGA GAT AAG TTG GAG	1155
Pro Met Leu Phe Thr Gly His Ser Leu Gly Arg Asp Lys Leu Glu	
340 345 350	
CAA CTG TTG GCA CAA GGT CGA AAG TCA AAG GAT GAA ATA AAC TCA	1200
Gln Leu Leu Ala Gln Gly Arg Lys Ser Lys Asp Glu Ile Asn Ser	
355 360 365	
ACC TAC AAG ATA ATG CGG AGA ATA GAG GCT GAA GAA TTA ACT CTT	1245
Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu Glu Leu Thr Leu	
370 375 380	
GAT GCT TCC GAA ATT GTC ATC ACT AGT ACA AGA CAG GAG ATT GAC	1290
Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg Gln Glu Ile Asp	
385 390 395	
GAG CAA TGG CGT TTG TAT GAT GGG TTT GAT CCA ATA TTA GAG CGT	1335
Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro Ile Leu Glu Arg	
400 405 410	
AAG TTA CGT GCA AGG ATC AAG CGC AAT GTC AGC TGT TAT GGC AGG	1380
Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser Cys Tyr Gly Arg	
415 420 425	
TTT ATG CCT CGT ATG GCT GTA ATT CCT CCT GGG ATG GAG TTC CAC	1425
Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly Met Glu Phe His	
430 435 440	
CAT ATT GTG CCA CAT GAA GGT GAC ATG GAT GGT GAA ACA GAA GGA	1470
His Ile Val Pro His Glu Gly Asp Met Asp Gly Glu Thr Glu Gly	
445 450 455	
AGT GAA GAT GGG AAG ACC CCG GAT CCA CCT ATT TGG GCA GAG ATT	1515
Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile Trp Ala Glu Ile	
460 465 470	

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ATG CGC TTC TTT TCT AAT CCA AGG AAG CCT ATG ATA CTC GCA CTT	1560
Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met Ile Leu Ala Leu	
475 480 485	
GCT AGG CCT GAT CCC AAG AAG AAC CTC ACT ACT TTA GTG AAA GCA	1605
Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr Leu Val Lys Ala	
490 495 500	
TTT GGT GAA TGT CGT CCA TTG AGA GAG CTT GCT AAT CTT ACT TTG	1650
Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu	
505 510 515	
ATA ATG GGT AAT CGA GAT AAT ATC GAC GAA ATG TCT AGC ACC AAT	1695
Ile Met Gly Asn Arg Asp Asn Ile Asp Glu Met Ser Ser Thr Asn	
520 525 530	
TCT GCA CTT CTT CTT TCA ATC TTG AAA ATG ATA GAT AAG TAT GAT	1740
Ser Ala Leu Leu Leu Ser Ile Leu Lys Met Ile Asp Lys Tyr Asp	
535 540 540	
CTT TAT GGT CAA GTA GCT TAT CCT AAA CAC CAC AAG CAG TCA GAT	1785
Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys Gln Ser Asp	
545 550 555	
GTT CCT GAT ATC TAC CGT CTT GCT GCA AAG ACT AAG GGT GTT TTT	1830
Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly Val Phe	
560 565 570	
ATT AAT CCA GCT TTT ATT GAG CCT TTT GGA CTG ACT TTG ATT GAG	1875
Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu Thr Leu Ile Glu	
575 580 585	
GCA GCA GCT TAT GGT CTC CCA ATG GTA GCC ACA AAA AAT GGA GGA	1920
Ala Ala Ala Tyr Gly Leu Pro Met Val Ala Thr Lys Asn Gly Gly	
590 595 600	

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CCT GTT GAT ATA CAT AGG GTT CTT GAC AAT GGT CTC TTA GTG GAT	1965
Pro Val Asp Ile His Arg Val Leu Asp Asn Gly Leu Leu Val Asp	
605 610 615	
CCC CAT GAT CAG CAG GCA ATT GCT GAT GCT CTT TTG AAG TTG GTT	2010
Pro His Asp Gln Gln Ala Ile Ala Asp Ala Leu Leu Lys Leu Val	
620 625 630	
GCT GAT AAG CAA CTG TGG GCT AAA TGC AGG GCA AAT GGA TTA AAA	2055
Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala Asn Gly Leu Lys	
635 640 645	
AAT ATC CAC CTT TTC TCA TGG CCC GAG CAC TGT AAA ACT TAT CTA	2100
Asn Ile His Leu Phe Ser Trp Pro Glu His Cys Lys Thr Tyr Leu	
650 655 660	
TCC CGG ATA GCT AGC TGC AAA CCA AGG CAA CCA CGC TGG CTG AGA	2145
Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln Pro Arg Trp Leu Arg	
665 670 675	
TCC ATT GAT GAT GAT GAT GAA AAT TCA GAA ACA GAT TCA CCT AGT	2190
Ser Ile Asp Asp Asp Asp Glu Asn Ser Glu Thr Asp Ser Pro Ser	
680 685 690	
GAT TCC TTG AGA GAT ATT CAT GAT ATA TCT CTG AAT TTG AGA TTT	2235
Asp Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu Arg Phe	
695 700 705	
TCA TTA GAT GGG GAA AAG AAT GAC AAT AAA GAA AAT GCT GAT AAT	2280
Ser Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala Asp Asn	
710 715 720	
ACA TTA GAC CCC GAA GTT CGA AGG AGC AAG TTA GAG AAT GCT GTT	2325
Thr Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn Ala Val	
725 730 735	

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20

TTG TCC TTA TCT AAG GGT GCA CTG AAG AGC ACA TCA AAA TCT TGG	2370
Leu Ser Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys Ser Trp	
740 745 750	
TCG TCA GAC AAG GCA GAC CAA AAC CCT GGT GCT GGT AAA TTC CCA	2415
Ser Ser Asp Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys Phe Pro	
755 760 765	
GCG ATT AGG AGG AGG CGA CAT ATT TTT GTT ATT GCA GTG GAT TGT	2460
Ala Ile Arg Arg Arg Arg His Ile Phe Val Ile Ala Val Asp Cys	
770 775 780	
GAT GCT AGC TCA GGA CTC TCT GGA AGT GTG AAA AAG ATA TTT GAG	2505
Asp Ala Ser Ser Gly Leu Ser Gly Ser Val Lys Lys Ile Phe Glu	
785 790 795	
GCT GTA GAG AAG GAA AGG GCA GAG GGT TCC ATT GGA TTT ATC CTG	2550
Ala Val Glu Lys Glu Arg Ala Glu Gly Ser Ile Gly Phe Ile Leu	
800 805 810	
GCT ACA TCT TTC AAT ATA TCA GAA GTA CAG TCT TTC CTG CTT TCA	2595
Ala Thr Ser Phe Asn Ile Ser Glu Val Gln Ser Phe Leu Leu Ser	
815 820 825	
GAG GGC ATG AAT CCT ACT GAT TTT GAT GCT TAC ATA TGC AAT AGT	2640
Glu Gly Met Asn Pro Thr Asp Phe Asp Ala Tyr Ile Cys Asn Ser	
830 835 840	
GGT GGT GAT CTT TAT TAT TCG TCC TTC CAT TCT GAG CAA AAT CCT	2685
Gly Gly Asp Leu Tyr Tyr Ser Ser Phe His Ser Glu Gln Asn Pro	
845 850 855	
TTT GTA GTT GAC TTG TAC TAT CAC TCA CAT ATT GAG TAT CGT TGG	2730
Phe Val Val Asp Leu Tyr Tyr His Ser His Ile Glu Tyr Arg Trp	
860 865 870	

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21

GGG GGC GAA GGA TTG AGA AAG ACT TTG GTG CGT TGG GCC GCC TCT	2775
Gly Gly Glu Gly Leu Arg Lys Thr Leu Val Arg Trp Ala Ala Ser	
875 880 885	
ATC ATT GAT AAG AAT GGT GAA AAT GGA GAT CAC ATT GTT GTT GAG	2820
Ile Ile Asp Lys Asn Gly Glu Asn Gly Asp His Ile Val Val Glu	
890 895 900	
GAT GAA GAC AAT TCA GCT GAC TAC TGC TAT ACT TTC AAA GTC TGC	2865
Asp Glu Asp Asn Ser Ala Asp Tyr Cys Tyr Thr Phe Lys Val Cys	
905 910 915	
AAG CCT GGG ACG GTT CCT CCA TCT AAA GAG CTT AGA AAA GTA ATG	2910
Lys Pro Gly Thr Val Pro Pro Ser Lys Glu Leu Arg Lys Val Met	
920 925 930	
CGA ATT CAG GCA CTT CGT TGT CAC GCT GTT TAT TGT CAA AAT GGG	2955
Arg Ile Gln Ala Leu Arg Cys His Ala Val Tyr Cys Gln Asn Gly	
935 940 945	
AGT AGG ATT AAT GTG ATC CCT GTA CTG GCA TCT CGG TCC CAA GCA	3000
Ser Arg Ile Asn Val Ile Pro Val Leu Ala Ser Arg Ser Gln Ala	
950 955 960	
CTC AGG TAC TTA TAT CTG CGA TGG GGA ATG GAC TTG TCG AAG TTG	3045
Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp Leu Ser Lys Leu	
965 970 975	
GTG GTT TTC GTC GGA GAA AGT GGT GAT ACC GAT TAT GAA GGA TTA	3090
Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu Gly Leu	
980 985 990	
ATC GGT GGT CTA CGC AAG GCT GTC ATA ATG AAA GGC CTC TGC ACT	3135
Ile Gly Gly Leu Arg Lys Ala Val Ile Met Lys Gly Leu Cys Thr	
995 1000 1005	

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22

AAT GCA AGC AGC TTA ATT CAC GGT AAT AGG AAT TAC CCG CTA TCT 3180
 Asn Ala Ser Ser Leu Ile His Gly Asn Arg Asn Tyr Pro Leu Ser
 1010 1015 1020

GAT GTT TTA CCA TTC GAC AGC CCT AAT GTC ATC CAA GCG GAC GAG 3225
 Asp Val Leu Pro Phe Asp Ser Pro Asn Val Ile Gln Ala Asp Glu
 1025 1030 1035

GAA TGT AGC AGC ACC GAA ATC CGT TGC TTA CTG GAG AAA CTA GCG 3270
 Glu Cys Ser Ser Thr Glu Ile Arg Cys Leu Leu Glu Lys Leu Ala
 1040 1045 1050

GTA CTC AAA GGA TAA TACCCTTCCC CCTTTGATTG TCAAAAACCT 3315
 Val Leu Lys Gly End
 1054

ATATGAGCTA TAAGACTATG CCATGAAAAG AATGGCCATC CATTTGGCTT GTCTTTTGAA 3375

GCTGTTAATA CTTTTCAACA GACTACAAA TGAGATGAGT CCTTTGATCC TCTTTAAAGG 3435

ACATAAAAGC TTTATGCAAG AACCAGTGCT GTAAAGTTAT AGAATTTCTT TTGCTATATA 3495

TGACATTCGA CAGAACCAGT TCCGGTTCAT CGAGAAAAAG AAATAAATTT CAACTTATAA 3555

ACATGCCTGA TCATGTAAAT TATCATATAC ATCCATCGGA AGGCATTATC GATGGGTTAT 3615

CAGATTTTTT 3625

SPS 3 sequence (Seq. ID No.3)

ATTTTTT TCTCTAAATT CTCTCTCACT GTCCTTATCA TTTCACCACC TCCATAAATC 57

TAGAAACATC TTTTCTATTC CGTTAATCTC TCTAGCACAC GCGGAGTGC GCGGAGGAG 117

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23

ATG GCG GGA AAC GAC TGG ATT AAC AGT TAC TTA GAG GCG ATA CTG 162
 Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu
 1 5 10 15

GAT GTA GGA CCA GGG CTA GAT GAT AAG AAA TCA TCG TTG TTG TTG 207
 Asp Val Gly Pro Gly Leu Asp Asp Lys Lys Ser Ser Leu Leu Leu
 20 25 30

AGA GAA AGA GGG AGG TTT AGT CCG ACG AGG TAC TTT GTT GAG GAA 252
 Arg Glu Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu
 35 40 45

GTT ATT ACT GGA TTC GAT GAG ACT GAT TTG CAT CGC TCG TGG ATC 297
 Val Ile Thr Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Ile
 50 55 60

CGA GCA CAA GCT ACT CGG AGT CCG CAG GAG AGG AAT ACT AGG CTC 342
 Arg Ala Gln Ala Thr Arg Ser Pro Gln Glu Arg Asn Thr Arg Leu
 65 70 75

GAG AAT ATG TGC TGG AGG ATT TGG AAT TTG GCT CGC CAG AAA AAG 387
 Glu Asn Met Cys Trp Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys
 80 85 90

CAG CTT GAG GGA GAG CAA GCT CAG TGG ATG GCA AAA CGC CGT CAA 432
 Gln Leu Glu Gly Glu Gln Ala Gln Trp Met Ala Lys Arg Arg Gln
 95 100 105

GAA CGT GAG AGA GGT CGC AGA GAA GCA GTT GCT GAT ATG TCA GAG 477
 Glu Arg Glu Arg Gly Arg Arg Glu Ala Val Ala Asp Met Ser Glu
 110 115 120

GAT CTA TCT GAG GGA GAG AAA GGA GAT ATA GTC GCT GAC ATG TCA 522
 Asp Leu Ser Glu Gly Glu Lys Gly Asp Ile Val Ala Asp Met Ser
 125 130 135

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24

TCT CAT GGT GAA AGT ACC AGA GGC CGA TTG CCT AGA ATC AGT TCT 567
 Ser His Gly Glu Ser Thr Arg Gly Arg Leu Pro Arg Ile Ser Ser
 140 145 150

GTT GAG ACA ATG GAA GCA TGG GTC AGT CAG CAG AGA GGA AAG AAG 612
 Val Glu Thr Met Glu Ala Trp Val Ser Gln Gln Arg Gly Lys Lys
 155 160 165

CTT TAT ATC GTG CTT ATA AGT TTA CAT GGT TTA ATT CGG GGT GAG 657
 Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu Ile Arg Gly Glu
 170 175 180

AAT ATG GAG CTT GGA CGG GAT TCT GAT ACT GGT GGT CAG GTG AAG 702
 Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys
 185 190 195

TAT GTA GTT GGA GCA ACT GTT GCA CAA GGT CGT TTG TCA AAG GAT 747
 Tyr Val Val Gly Ala Thr Val Ala Gln Gly Arg Leu Ser Lys Asp
 200 205 210

GAA ATA AAC TCA ACC TAC AAG ATA ATG CGG AGA ATA GAG GCT GAA 792
 Glu Ile Asn Ser Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu
 215 220 225

GAA TTA ACT CTT GAT GCT TCC GAA ATT GTC ATC ACT AGT ACA AGA 837
 Glu Leu Thr Leu Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg
 230 235 240

CAG GAG ATT GAC GAG CAA TGG CGT TTG TAT GAT GGG TTT GAT CCA 882
 Gln Glu Ile Asp Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro
 245 250 255

ATA TTA GAG CGT AAG TTA CGT GCA AGG ATC AAG CGC AAT GTC AGC 927
 Ile Leu Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser
 260 265 270

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25

TGT TAT GGC AGG TTT ATG CCT CGT ATG GCT GTA ATT CCT CCT GGG 972
 Cys Tyr Gly Arg Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly
 275 280 285

ATG GAG TTC CAC CAT ATT GTG CCA CAT GAA GGT GAC ATG GAT GGT 1017
 Met Glu Phe His His Ile Val Pro His Glu Gly Asp Met Asp Gly
 290 295 300

GAA ACA GAA GGA AGT GAA GAT GGA AAG ACC CCG GAT CCA CCT ATT 1062
 Glu Thr Glu Gly Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile
 305 310 315

TGG GCA GAG ATT ATG CGC TTC TTT TCT AAT CCA AGG AAG CCT ATG 1107
 Trp Ala Glu Ile Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met
 320 330 335

ATA CTC GCA CTT GCT AGG CCT GAT CCC AAG AAG AAC CTC ACT ACT 1152
 Ile Leu Ala Leu Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr
 340 345 350

TTA GTG AAA GCA TTT GGT GAA TGT CGT CCA TTG AGA GAC CTT GCT 1197
 Leu Val Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Asp Leu Ala
 355 360 365

AAT CTT ACT TTG ATA ATG GGT AAT CGA GAT AAT ATC GAC GAA ATG 1242
 Asn Leu Thr Leu Ile Met Gly Asn Arg Asp Asn Ile Asp Glu Met
 370 375 380

TCT AGC ACC AAT TCT GCA CTT CTT CTT TCA ATC TTG AAG ATG ATA 1287
 Ser Ser Thr Asn Ser Ala Leu Leu Leu Ser Ile Leu Lys Met Ile
 385 390 395

GAT AAG TAT GAT CTT TAT GGT CTA GTA GCT TAT CCT AAA CAC CAC 1332
 Asp Lys Tyr Asp Leu Tyr Gly Leu Val Ala Tyr Pro Lys His His
 400 405 410

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26

AAG CAG TCA GAT GTT CCT GAT ATC TAC CGT CTT GCT GCA AAG ACT 1377
 Lys Gln Ser Asp Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr
 415 420 425

AAG GGT GTT TTT ATT AAT CCA GCT TTT ATT GAG CCT TTT GGA CTG 1422
 Lys Gly Val Phe Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu
 430 435 440

ACT TTG ATT GAG GCA GCA GCT TAT GGT CTC CCA ATG GTA GCC ACA 1467
 Thr Leu Ile Glu Ala Ala Ala Tyr Gly Leu Pro Met Val Ala Thr
 445 450 455

AAA AAT GGA GGA CCT GTT GAT ATA CAT AGG GTT CTT GAC AAT GGT 1512
 Lys Asn Gly Gly Pro Val Asp Ile His Arg Val Leu Asp Asn Gly
 460 465 470

CTC TTA GTG GAT CCC CAT GAT CAG CAG GCA ATT GCT GAT GCT CTT 1557
 Leu Leu Val Asp Pro His Asp Gln Gln Ala Ile Ala Asp Ala Leu
 475 480 485

TTG AAG TTG GTT GCT GAT AAG CAA CTG TGG GCT AAA TGC AGG GCA 1602
 Leu Lys Leu Val Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala
 490 495 500

AAT GGA TTA AAA AAT ATC CAC CTT TTC TCA TGG CCC GAG CAC TGT 1647
 Asn Gly Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His Cys
 505 510 515

AAA ACT TAT CTA TCC CGG ATA GCT AGC TGC AAA CCG AGG CAA CAT 1692
 Lys Thr Tyr Leu Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln His
 520 525 530

TCC TTG AGA GAT ATT CAT GAT ATA TCT CTG AAT TTG AGA TTT TCA 1737
 Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu Arg Phe Ser
 535 540 540

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TTA GAT GGG GAA AAG AAT GAC AAT AAA GAA AAT GCT GAT AAT ACA	1782
Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala Asp Asn Thr	
545 550 555	
TTA GAC CCC GAA GTT CGA AGG AGC AAG TTA GAG AAT GCT GTT TTG	1827
Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn Ala Val Leu	
560 565 570	
TCC TTA TCT AAG GGT GCA CTG AAG AGC ACA TCA AAA TCT TGG TCG	1872
Ser Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys Ser Trp Ser	
575 580 585	
TCA GAC AAG GCA GAC CAA AAT CCT GGT GCT GGT AAA TTC CCA GCG	1917
Ser Asp Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys Phe Pro Ala	
590 595 600	
ATT AGG AGG AGG CGA CAT ATT TTT GTT ATT GCA GTG GAT TGT GAT	1962
Ile Arg Arg Arg Arg His Ile Phe Val Ile Ala Val Asp Cys Asp	
605 610 615	
GCT AGC TCA GGA CTC TCT GGA AGT ATG AAA AAG ATA TTT GAG GCT	2007
Ala Ser Ser Gly Leu Ser Gly Ser Met Lys Lys Ile Phe Glu Ala	
620 625 630	
GTA GAG AAG GAA AGG GCA GAG GGT TCC ATT GGA TTT ATC CTT GCT	2052
Val Glu Lys Glu Arg Ala Glu Gly Ser Ile Gly Phe Ile Leu Ala	
635 640 645	
ACA TCT TTC AAT ATA TCA GAA GTA CAG TCT TTC CTG CTT TCA GAG	2097
Thr Ser Phe Asn Ile Ser Glu Val Gln Ser Phe Leu Leu Ser Glu	
650 655 660	
GGC ATG AAT CCT ACT GAG CAA AAT CCT TTT GTA GTT GAC TTG TAC	2142
Gly Met Asn Pro Thr Glu Gln Asn Pro Phe Val Val Asp Leu Tyr	
665 670 675	

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28

TAT CAC TCA CAT ATT GAG TAT CGT TGG GGG GGC GAA GGG TTG AGA	2187
Tyr His Ser His Ile Glu Tyr Arg Trp Gly Gly Glu Gly Leu Arg	
680 685 690	
AAG ACT TTG GTG CGT TGG GCC GCC TCT ATC ATT GAT AAG AAT GGT	2232
Lys Thr Leu Val Arg Trp Ala Ala Ser Ile Ile Asp Lys Asn Gly	
695 700 705	
GAA AAT GGA GAT CAC ATT GTT GTT GAG GAT GAA GAC AAT TCA GCT	2277
Glu Asn Gly Asp His Ile Val Val Glu Asp Glu Asp Asn Ser Ala	
710 715 720	
GAC TAC TGC TAT ACA TTC AAA GTT TGC AAG CCT GGG ACG GTT CCT	2322
Asp Tyr Cys Tyr Thr Phe Lys Val Cys Lys Pro Gly Thr Val Pro	
725 730 735	
CCA TCT AAA GAA CTT AGA AAA GTA ATG CGA ATT CAG GCA CTT CGT	2367
Pro Ser Lys Glu Leu Arg Lys Val Met Arg Ile Gln Ala Leu Arg	
740 745 750	
TGT CAC GCT GTT TAT TGT CAA AAT GGG AGT AGG ATT AAT GTG ATC	2412
Cys His Ala Val Tyr Cys Gln Asn Gly Ser Arg Ile Asn Val Ile	
755 760 765	
CCT GTA CTG GCA TCT CGG TCC CAA GCA CTC AGG TAC TTA TAT CTG	2457
Pro Val Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Leu	
770 775 780	
CGA TGG GGA ATG GTC CCT GTA CTG GCA TCT CGG TCC CAA GCA CTC	2502
Arg Trp Gly Met Val Pro Val Leu Ala Ser Arg Ser Gln Ala Leu	
785 790 795	
AGG TAC TTA TAT CTG CGA TGG GGA ATG GTC CCT GTA CTG GCA TCT	2547
Arg Tyr Leu Tyr Leu Arg Trp Gly Met Val Pro Val Leu Ala Ser	
800 805 810	

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29

CGG TCC CAA GCA CTC AGG TAC TTA TAT CTG CGA TGG GGA ATG GAC 2592
 Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp
 815 820 825

TTG TCG AAG TTG GTG GTT TTC GTC GGA GAA AGT GGT GAT ACC GAT 2637
 Leu Ser Lys Leu Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp
 830 835 840

TAT GAA GGA TTG ATC GGT GGT CTA CGC AAG GCT GTC ATA ATG AAA 2682
 Tyr Glu Gly Leu Ile Gly Gly Leu Arg Lys Ala Val Ile Met Lys
 845 850 855

GGA CTC TGC ACT AAT GCA AGC AGC TTA ATT CAC GGT AAT AGG AAT 2727
 Gly Leu Cys Thr Asn Ala Ser Ser Leu Ile His Gly Asn Arg Asn
 860 865 870

TAC CCG CTA TCT GAT GTT TTA CCA TTC GAG AGC CCT AAT GTC ATC 2772
 Tyr Pro Leu Ser Asp Val Leu Pro Phe Glu Ser Pro Asn Val Ile
 875 880 885

CAA GCG GAT GAG GAA TGT AGC AGC ACC GGA ATC CGT TCC TTA CTG 2817
 Gln Ala Asp Glu Glu Cys Ser Ser Thr Gly Ile Arg Ser Leu Leu
 905 910 915

GAG AAA CTA GCG GTA CTC AAA GGA TAA TACCCTTCCC CCTTTGATTG 2864
 Glu Lys Leu Ala Val Leu Lys Gly End
 920

TCAAAAACCT ATATGAGCTA AGATTATGCC ATGAAAAGAA TGGCCATCCA TTTGGCTTGT2924

CTTTTG 2930

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All sequences are cDNA sequences and stem from a cDNA library of leaf tissue. The expression gene is the same in various plant tissues. As promoter, there can generally be used any promoter which is active in plants. The promoter should ensure that the foreign gene is expressed in the plant. The promoter can be so chosen that the expression occurs only in specified tissues, at a determined time point in the plant's development or at a time point determined by outside influences. The promoter can be homologous or heterologous to the plant. Suitable promoters are e.g. the promoter of the 35S RNA of the cauliflower mosaic virus, the patatin promoter B33 (Rocha-Sosa et al. (1989) EMBO J 8: 23-29) or a promoter that ensures an expression only in photosynthetically active tissues. Other promoters can be used which ensure an expression only in specified organs, such as the root, tuber, seed, stem or specified cell types such as mesophyll, epidermal or transport cells. For hindering cold sweetening, suitable promoters are those which ensure an activation of the transcription is stored in harvested parts of the plants. For this, there can be considered cold induced promoters or such promoters, that become active during the transition of the tuber from the phase where it stores material to the phase where it gives up material.

The coding sequence contains the information for the formation of an mRNA for the sucrose-phosphate-synthase or for the formation of an anti-sense RNA for the SPS. whether the translatable mRNA or an anti-sense RNA is formed, depends on the orientation of the coding sequence in relation to the promoter. If the 3' end of the coding sequence is fused to the 3' end of the promoter, an anti-sense RNA results, and by fusion of the 5' end of the coding to the 3' end of the promoter a translatable RNA

results. This latter leads to an increase of the SPS activity in the cell, whilst the first leads to a reduction of the SPS activity in the cell. Such a reduction of SPS activity is of especial significance in view of the undesirable formation of sucrose and/or reducing sugars as a result of cold storage of harvested organs.

The coding sequence for SPS can be one of the three described above or one that is derived by modifications of the sequences described above. A derivation can be carried out, e.g. by current methods of mutagenesis and/or recombination. For this especially, changes of SPS sequences are envisaged, that lead to a neutralisation of the plant's own regulation mechanism.

The DNA sequences of the invention can be used for the preparation of derivatives whose gene products are not subjected to the plant's own activity regulation during a phosphorylation reaction.

Further, the sequences can also be used for the preparation of derivatives by targeted and non-targeted mutagenesis.

The invention relates further to derivatives of the DNA sequences of the invention that are obtained by exchange of single bases or by deletion or insertion of base sequences and which code for proteins with a comparable activity to sucrose-phosphate-synthase.

The 5' untranslated area of the sequence Seq. ID No 1 definitely does not belong to SPS, but is added as a cloning artefact. The methionine start codon of the coding region lies in a region in which no homology of the amino

acid sequence to the other SPS sequences is involved.
Since this sequence does not also fully coincide in the
homologous region with one of the other sequences, it is
recognisable that the sequence Seq. ID No 1 is not a
5 derivative of the sequences Seq. ID No 2 and Seq. ID No 3.

The termination sequence provides the correct finishing of
the transcription and the attachment of a polyadenyl group
to the RNA. This polyadenyl group has an important
10 function in the stabilisation of RNA molecules in the
cells. With suitable plasmids, which contain the DNA
sequences of the invention, plants can be transformed with
the object of raising and/or reducing the SPS activity
and/or the modification of the sucrose concentration.

15 Plasmids, that can be used are e.g. p35S-anti-pot-SPS (DSM
7125) and pB33-anti-pot-SPS (DSM 7124). With the gene
35S-anti-pot-SPS, located on the plasmid
p35S-anti-pot-SPS, the concentration of the mRNA for the
20 SPS protein and the enzymatic activity, for example, can
be reduced. With the gene B33S-anti-pot-SPS, located on
the plasmid pB33-anti-pot-SPS, the concentration of the
mRNA for the SPS protein and the enzymatic activity,
specifically for potato tubers for example, can be
25 reduced. In a similar way to the SPS sequence (Seq. ID No.
1) located on this plasmid, other SPS sequences, e.g. the
sequences Seq. ID No. 2 and Seq. ID No. 3 also be cloned
in suitable vectors and for the same purpose.

30 In the plant, the SPS is subjected to an activity control
by phosphorylation. This allows the plant to regulate the
activity of the enzyme within a fixed frame independent of
the amount of the SPS protein. If one of the changes
occurring outside the activity of the SPS is to achieved,
35 it is necessary to evade the plant's own regulation

mechanism. Therefore changing the phosphorylation possibilities is an important target for influencing the SPS activity and thus the sucrose content of the plant.

- 5 It is not known, in which position in the SPS protein, target directed changes of the coding regions can be achieved, which serve the purpose of introducing in the plant, SPS activity, which is not subject to any of the plant's own controls.

10

- The DNA sequence described here, which contains the coding region for SPS from *Solanum tuberosum*, allows the identification of the sites of protein phosphorylation of the SPS. By using standard methods (Sambrook, J., Fritsch, 15 E. F., Maniatis, T. (1989) Molecular Cloning: A laboratory Manual, 2nd. Edn., Cold Spring Harbor Laboratory Press, NY, USA), a localisation of the phosphorylation positions of SPS is possible using the DNA sequences of the invention. These being known, by use of the plasmids with 20 the SPS sequence, a target directed mutagenesis (Sambrook et al, 1989) of the coding region of SPS and/or a non-target directed mutagenesis (Sambrook et al, 1989) and subsequent probing of the desired mutations of the coding region of the SPS can be undertaken. Derivatives of the 25 coding region can be prepared with the help of this plasmid, whose derived proteins are not subjected to the plants own regulation mechanisms.

- Since the SPS enzyme is regulated by phosphorylation in 30 all tested species, except maize, one can refer to sequence comparisons, to identify possible phosphorylation sites. The criterium for this is that a serine residue appears in an acidic medium in the regulated SPS protein, but not however with maize.

35

There are 12 such serine residues in the sequences Seq. ID No. 2 and Seq ID No. 3. In the sequence Seq ID No. 1, the first of the 12 serine residues is missing, since the coding region begins just later. The sequence Seq. ID No. 1 is thus especially suitable for the production of an SPS activity in plants, that is not liable to endogenous activity regulation.

For the introduction of the SPS sequence in higher plants, a large number of cloning vectors are available, which contain a replication signal for *E. coli* and a marker, which allows a selection of the transformed cells. Examples of vectors are pBR 322, pUC-series, M13 mp-series, pACYC 184; EMBL 3 etc.. According to the introduction method of the desired gene in the plant, other DNA sequences may be suitable. Should the Ti- or Ri-plasmid be used, e.g. for the transformation of the plant cell, then at least the right boundary, often however both the right and left boundary of the Ti- and Ri-Plasmid T-DNA, is attached, as a flanking region, to the gene being introduced. The use of T-DNA for the transformation of plants cells has been intensively researched and is well described in EP 120 516; Hoekama, In: The Binary Plant Vector System, Offset-drukkerij Kanters B.V. Alblasserdam, (1985), Chapter V; Fraley, et al., Crit. Rev. Plant Sci., 4:1-46 and An et al. (1985) EMBO J. 4: 277-287. Once the introduced DNA is integrated in the genome, it is as a rule stable there and remains also in the offspring of the original transformed cells. It normally contains a selection marker, which induces resistance in the transformed plant cells against a biocide or antibiotic such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricin etc. The individual marker employed should therefore allow the selection of transformed cells from cells, which lack the introduced

DNA.

For the introduction of DNA into a plant, besides transformation using *Agrobacteria*, there are many other techniques available. These techniques include the fusion of protoplasts, microinjection of DNA and electroporation, as well as ballistic methods and virus infection. From the transformed plant material, whole plants can be regenerated in a suitable medium, which contains antibiotics or biocides for the selection. The resulting plants can then be tested for the presence of introduced DNA. No special demands are placed on the plasmids in injection and electroporation. Simple plasmids, such as e.g. pUC-derivatives can be used. Should however whole plants be regenerated from such transformed cells the presence of a selectable marker gene is necessary. The transformed cells grow within the plants in the usual manner (see also McCormick et al. (1986) Plant Cell Reports 5: 81-84). These plants can be grown normally and crossed with plants, that possess the same transformed genes or different. The resulting hybrid individuals have the corresponding phenotypical properties.

Deposits

25

The following plasmids were deposited at the Deutschen Sammlung von Mikroorganismen (DSM) in Braunschweig, Germany on the 12.06.1992 (deposit number):

30	Plasmid p35S-anti-pot-SPS	(DSM 7125)
	Plasmid pB33-anti-pot-SPS	(DSM 7124)

Description of the Figures

Fig. 1: Structure of the 35S-anti-pot-SPS gene

- 5 A = Fragment A: CaMV 35S promoter, nt 6909-7437 (Franck
 et al., 1980, Cell 21: 285-294)
- B = Fragment B: sucrose phosphate synthase, EcoRV
 Fragment (nt 1 bis 2011), ca. 2000 bp, orientation:
 anti-sense
- 10 C = Fragment C: nt 11748-11939 of the T-DNA of the
 Ti-plasmid pTiACH5; Gielen et al., 1984, EMBO J 3:
 835-846)

Fig. 2: Structure of the B33-anti-pot-SPS gene

- 15 A = Fragment A: B33 promoter of the patatin gene from *S.*
 tuberosum, (Rocha-Sosa et al., 1989, EMBO J 8:
 23-29), ca 530 bp
- B = Fragment B: sucrose phosphate synthase (s. Fig. 2),
 EcoRV fragment (nt 2011 bis 1), ca. 2000 bp,
20 orientation: anti-sense
- C = Fragment C: nt 11748-11939 of T-DNA of the
 Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J 3:
 835-846)

- 25 Fig. 3: shows the results of the transformation of
 transgenic potato plants.

Control = wild type plants

1-75 = individual transgenic plants

30

- Fig. 4: shows the results of the transformation of
 potato plants

Control = wild type plants

35 3 - 20 = individual transgenic plants

In order to understand the examples forming the basis of this invention all the processes necessary for these tests and which are known per se will first of all be listed:

5 1. Cloning process

The vectors pUC 18/19 and M13mp10 series (Yanisch-Perron et al. (1985) Gene 33: 103-119), as well as the vector EMBL 3 (Frischauf et al. (1983) J Mol Biol 170: 827- 842) were used for cloning.

10

For the plant transformations, the gene constructs were cloned in the binary vector BIN 19 (Bevan (1984) Nucl. Acids Res 12: 8711-8720)

15

2. Bacterial strains

The *E. coli* strain BMH71-18 (Messing et al., Proc. Natl. Acad. Sci. USA (1977), 24, 6342-6346) or TB1 was used for the pUC and M13 mP vectors.

20

For the vector BIN19, the *E. coli* strain TB1 exclusively, was used. TB1 is a recombinant-negative, tetracycline-resistant derivative of strain JM101 (Yanisch-Perron et al., Gene (1985), 33, 103-119). The genotype of the TB1 strain is (Bart Barrel, personal communication):
F'(traD36, proAB, lacI, lacZAM15), Δ(lac, pro), SupE, thiS, recA, Srl::Tn10(TcR).

25

The transformation of the plasmids into the potato plants was carried out using *Agrobacterium tumefaciens* strain LBA4404 (Bevan, (1984), Nucl. Acids Res. 12, 8711-8720).

30

3. Transformation of *Agrobacterium tumefaciens*

35 In the case of BIN19 derivatives, the insertion of the DNA

into the *Agrobacterium* was effected by direct transformation in accordance with the method of Holsters et al., (1978) (Mol Gene Genet 163: 181-187). The plasmid DNA of the transformed *Agrobacterium* was isolated in accordance with the method of Birnboim and Doly (1979) (Nucl Acids Res 7: 1513-1523) and was analysed by gel electrophoresis after suitable restriction cleavage.

4. Plant transformation

Ten small leaves, wounded with a scalpel, of a sterile potato culture were placed in 10 ml of MS medium with 2% sucrose containing 30-50 μ l of an *Agrobacterium tumefaciens* overnight culture grown under selection. After 3-5 minutes gentle shaking, the leaves were laid out on MS medium of 1.6% glucose, 2 mg/l of zeatin ribose, 0.02 mg/l of naphthylacetic acid, 0.02 mg/l of gibberellic acid, 500 mg/l of claforan, 50 mg/l of kanamycin and 0.8% bacto agar. After incubation for one week at 25°C and 3000 lux, the claforan concentration in the medium was reduced by half.

5. SPS activity test

The SPS activity was determined according to the method of Siegel and Stitt (1990, Plant Science 66: 205-210) in a two stage analysis. To 180 μ l of a solution of 50mM HEPES/KOH (pH 7.4), 5mM magnesium chloride, 5mM fructose-6-phosphate, 25mM glucose-6-phosphate and 6mM uridine-5'-diphosphoglucose 20 μ l of probe was added and incubated for 10 minutes at 25°C. It was heated for 3 minutes at 95°C, to complete the reaction. After centrifuging, the supernatant was spectroscopically analysed for the liberation of uridine-5'-diphosphate, whereby a pyruvate-kinase coupling enzyme reaction was used. Preparations without hexose phosphate, as well as

the measurement of the recovery of added uridine-5'-diphosphate act as controls.

ExamplesExample 1

- 5 Cloning of genes of the sucrose-phosphate-synthase from potato

Poly-A⁺ RNA was isolated from large leaves of spinach plants as well as potato plants grown in the greenhouse.

10 Resulting from the poly-A⁺ RNA, a cDNA library in the expression vector Lambda Zap II was laid out. 100,000 Plaques of both libraries were separated from spinach using a rabbit antiserum directed against pure SPS protein in relation to immunologically cross reacting protein.

15 (Sonnewald et al., 1992, in press). From the potato library, positively reacting clones were obtained. These clones were further purified by standard methods and, by in vivo excision, plasmids were obtained which carried a double stranded cDNA as an insertion. After testing the

20 size of the insertions, individual clones were analysed by determining the primary sequence.

Example 2

- 25 Determination of the nucleotide sequence of the SPS from potato coding cDNA molecules and derivation of the corresponding amino acid sequences

The nucleotide sequences of the insertions obtained from

30 Example 1, were determined by standard methods by means of the dideoxy method (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA, 74, 5463-5467). The nucleotide sequences (Seq. ID No. 1 to Seq. ID No. 3) are described above. The amino acid sequences derived therefrom are also given.

Example 3Construct of the plasmid p35s-anti-pot-SPS and insertion of gene 35s-anti-pot-sps in the genome of potato plants

5

The gene 35s-anti-pot-SPS consists of the three fragments A, B and C (see Fig 1).

The plasmid was prepared as follows:

10 From the pBluescript plasmid with the total insertion, an approximately 2 kb size fragment was prepared by EcoRV cleavage, and this was cloned in the SmaI cleavage site of the vector pBinAR (Höfgen & Willmitzer, 1990, Plant Sci., 66, 221-230). The vector pBinAR is a derivative of the
15 binary vector BIN 19 (Bevan, 1984, Nucl. Acids Res. 12: 8711-8721) and was transferred using an *Agrobacterium tumefaciens* mediated transformation into potato. Intact, fertile plants were regenerated from the transformed cells.

20

As a result of the transformation, some transgenic potato plants were shown to have a reduced amount of RNA coding for the potato SPS (see Fig. 3). 50 µg total RNA in a Northern blot experiment was hybridised with the probe for
25 SPS from potato.

Further the plants showed a reduction in SPS activity (see Table I).

30 Thus, by the transfer and expression of the gene 35s-anti-pot-SPS in potato plants, the amount of mRNA for the SPS protein which is formed, as well as the existing enzymatic activity can be significantly reduced.

35

Example 4Construct of plasmid pB33-anti-pot-SPS and insertion of gene B33-anti-pot-SPS in the genome of potato plants

5

The gene B33-anti-pot-SPS consists of the three fragments A, B and C (see Fig 4). The plasmid was prepared in an analogous method to that described in Example 3, except a pBin 19 derivative was used as starting vector, that
10 contains the B33 promoter of the patatin gene from *Solanum tuberosum* (Rocha-Sosa et al., 1989, EMBO J. 8: 23-29) in place of the 35S promoter of pBinAR.

The gene B33-anti-pot-SPS was transferred using an
15 *Agrobacterium tumefaciens* mediated transformation into potato. Intact, fertile plants were regenerated from the transformed cells.

As a result of the transformation, some transgenic potato
20 plants were shown with a reduced amount of RNA coding for the potato SPS (see Fig. 4). 50 µg total RNA in a Northern blot experiment was hybridised with the probe for SPS from potato.

25 Further the plants also showed a reduction of the SPS activity only in the tubers.

Thus, by the transfer and expression of the gene
35s-anti-pot-SPS in potato plants, the amount of mRNA for
30 the SPS protein which is formed, as well as the existing enzymatic activity can be significantly reduced.

Table I

Results of the transformation of potato plants

5	1	2	3	4	5
	Control	26.1	3.6	13.8	100
	1-55	11.8	2.7	22.9	45
	1-57	20.4	5.9	28.9	78
10	1-59	3.8	1.4	36.8	14.6
	1-67	3.8	1.7	44.7	14.6
	1-69	17.2	2.0	11.7	67
	1-72	14.6	1.9	13.0	56
	1-74	5.1	1.7	33.3	19.5

15

Column 1: Control = Wild type plants, numbers indicate individual transgenic plants

Column 2: Maximal speed of the enzyme reaction in the SPS activity test in nmol/min/mg.

20 Column 3: Speed in the SPS activity test in nmol/min/mg.

Column 4: Activity level of the SPS in %.

Column 5: Residual activity of the SPS in %.

CLAIMS

1. DNA sequence with the coding region for
sucrose-phosphate-synthase (SPS) from *Solanum*
5 *tuberosum* for the preparation of plants with
modified sucrose concentration, characterised in
that this sequence has the following nucleotide
sequence (Seq. ID No.1):

CTATTCTCTC CCCTCCTTTT TCTCCTCTCT TCAACCCCAA AACTTCCCTT TCAAAGCCTT 60
TGCTTTCCTT TTCTCACTTA CCCAGATCAA CTAAGCCAAT TTGCTGTAGC CTCAGAAAAC 120
AGCATTCCCA GATTGAAAAA GAATCTTTTT CAGTACCCAA AAGTTGGGTT TCTCATGTCC 180
AGCAAGGATT AGCTGCTCTA GCTATTTCTT TAGCCCTTAA TTTTGTCCA GTTGTGTCTT 240
CTGATTCTGC ATTGGCATCT GAATTTGATG TGTTAAATGA AGGGCCACCA AAGGACTCAT 300
ATGTAGTTGA TGATGCTGGT GTGCTTAGCA GGGTGACAAA GTCTGATTG AAGGCATTGT 360
TGTCTGATGT GGAGAAGAGA AAAGGCTTCC ACATTAATTT CATCACTGTC CGCAAGCTCA 420
CTAGCAAAGC TGATGCTTTT GAGTATGCTG ACCAAGTTTT GGAGAAGTGG TACCCTAGTG 480
TTGAACAAGG AAATGATAAG GGTATAGTTG TGCTTGTTAC AAGTCAAAAG GAAGCGCAA 540
TAACCGGTGG CCCTGATTTT GTAAAGGCCG TTGGAGATAC TGTCTTGAT GCTACCGTCT 600
CAGAGAACCT TCCAGTGTG GCTACTGAAG AGAAGTACAA TGAAGCAGTT TTCAGCACTG 660
CCACACGTCT TGTGTCAGCC ATTGATGGCC TTCCTGATCC TGGTGGACCC CAACTCAAGG 720
ATAACAAAAG AGAGTCCAAC TTCAAATCCA GAGAGGAAAC TGATGAGAAA AGAGGACAAT 780

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TCACACTTGT GGTGGGTGGG CTGTTAGTGA TTGCTTTTGT TGTTCCTATG GCTCAATACT 840

ATGCATATGT TTCAAAGAAG TGAAGTGTCT GATTCTGGAA AGTTACATTT TCGTGAGATT 900

TGAGTAAGCA TGTATATTAT CGTGACAAA ATGGTCCATT CGGAAATGAC TGATTC 956

ATG AGA TAT TTA AAA AGG ATA AAT ATG AAG ATT TGG ACC TCC CCT 1001

Met Arg Tyr Leu Lys Arg Ile Asn Met Lys Ile Trp Thr Ser Pro

1 5 10 15

AAC ATA ACG GAT ACT GCC ATT TCT TTT TCA GAG ATG CTG ACG CCA 1046

Asn Ile Thr Asp Thr Ala Ile Ser Phe Ser Glu Met Leu Thr Pro

20 25 30

ATA AGT ACA GAC GGC TTG ATG ACT GAG ATG GGG GAG AGT AGT GGT 1091

Ile Ser Thr Asp Gly Leu Met Thr Glu Met Gly Glu Ser Ser Gly

35 40 45

GCT TAT ATT ATT CGC ATT CCT TTT GGA CCA AGA GAG AAA TAT ATT 1136

Ala Tyr Ile Ile Arg Ile Pro Phe Gly Pro Arg Glu Lys Tyr Ile

50 55 60

CCA AAA GAA CAG CTA TGG CCC TAT ATT CCC GAA TTT GTT GAT GGT 1181

Pro Lys Glu Gln Leu Trp Pro Tyr Ile Pro Glu Phe Val Asp Gly

65 70 75

GCA CTT AAC CAT ATT ATT CAA ATG TCC AAA GTT CTT GGG GAG CAA 1226

Ala Leu Asn His Ile Ile Gln Met Ser Lys Val Leu Gly Glu Gln

80 85 90

ATT GGT AGT GGC TAT CCT GTG TGG CCT GTT GCC ATA CAC GGA CAT 1271

Ile Gly Ser Gly Tyr Pro Val Trp Pro Val Ala Ile His Gly His

95 100 105

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TAT GCT GAT GCT GGC GAC TCA GCT GCT CTC CTG TCA GGT GCT TTA	1316
Tyr Ala Asp Ala Gly Asp Ser Ala Ala Leu Leu Ser Gly Ala Leu	
110 115 120	
AAT GTA CCA ATG CTT TTC ACT GGT CAC TCA CTT GGT AGA GAT AAG	1361
Asn Val Pro Met Leu Phe Thr Gly His Ser Leu Gly Arg Asp Lys	
125 130 135	
TTG GAG CAA CTG TTG CGA CAA GGT CGT TTG TCA AAG GAT GAA ATA	1406
Leu Glu Gln Leu Leu Arg Gln Gly Arg Leu Ser Lys Asp Glu Ile	
140 145 150	
AAC TCA ACC TAC AAG ATA ATG CGG AGA ATA GAG GCT GAA GAA TTA	1451
Asn Ser Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu Glu Leu	
155 160 165	
ACT CTT GAT GCT TCC GAA ATT GTC ATC ACT AGT ACA AGA CAG GAG	1496
Thr Leu Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg Gln Glu	
170 175 180	
ATT GAC GAG CAA TGG CGT TTG TAT GAT GGG TTT GAT CCA ATA TTA	1541
Ile Asp Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro Ile Leu	
185 190 195	
GAG CGT AAG TTA CGT GCA AGG ATC AAG CGC AAT GTC AGC TGT TAT	1586
Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser Cys Tyr	
200 205 210	
GGC AGG TTT ATG CCT CGT ATG GCT GTA ATT CCT CCT GGG ATG GAG	1531
Gly Arg Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly Met Glu	
215 220 225	

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TTC CAC CAT ATT GTG CCA CAT GAA GGT GAC ATG GAT GGA GAA ACA	1676
Phe His His Ile Val Pro His Glu Gly Asp Met Asp Gly Glu Thr	
230 235 240	
GAA GGA AGT GAA GAT GGG AAG ACC CCG GAT CCA CCT ATT TGG GCA	1721
Glu Gly Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile Trp Ala	
245 250 255	
GAG ATT ATG CGC TTC TTT TCT AAT CCA AGG AAG CCT ATG ATA CTC	1766
Glu Ile Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met Ile Leu	
260 265 270	
GCA CTT GCT AGG CCT GAT CCC AAG AAG AAC CTC ACT ACT TTA GTG	1811
Ala Leu Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr Leu Val	
275 280 285	
AAA GCA TTT GGT GAA TGT CGT CCA TTG AGA GAG CTT GCT AAT CTT	1856
Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu	
290 295 300	
ACT TTG ATA ATG GGT AAT CGA GAT AAT ATC GAC GAA ATG TCT AGC	1901
Thr Leu Ile Met Gly Asn Arg Asp Asn Ile Asp Glu Met Ser Ser	
305 310 315	
ACC AAT TCT GCA CTT CTT CTT TCA ATC TTG AAA ATG ATA GAT AAG	1946
Thr Asn Ser Ala Leu Leu Leu Ser Ile Leu Lys Met Ile Asp Lys	
320 325 330	
TAT GAT CTT TAT GGT CAA GTA GCT TAT CCT AAA CAC CAC AAG CAG	1991
Tyr Asp Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys Gln	
335 340 345	
TCA GAT GTT CCT GAT ATC TAC CGT CTT GCT GCA AAG ACT AAG GGT	2036
Ser Asp Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly	
350 355 360	

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GTT TTT ATT AAT CCA GCT TTT ATT GAG CCT TTT GGA CTG ACT TTG	2081
Val Phe Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu Thr Leu	
365 370 375	
ATT GAG GCA GCA GCT TAT GGT CTC CCA ATG GTA GCC ACA AAA AAT	2126
Ile Glu Ala Ala Ala Tyr Gly Leu Pro Met Val Ala Thr Lys Asn	
380 385 390	
GGA GGA CCT GTT GAT ATA CAT AGG GTT CTT GAC AAT GGT CTC TTA	2171
Gly Gly Pro Val Asp Ile His Arg Val Leu Asp Asn Gly Leu Leu	
395 400 405	
GTG GAT CCC CAT GAT CAG CAG GCA ATT GCT GAT GCT CTT TTG AAG	2216
Val Asp Pro His Asp Gln Gln Ala Ile Ala Asp Ala Leu Leu Lys	
410 415 420	
TTG GTT GCT GAT AAG CAA CTG TGG GCT AAA TGC AGG GCA AAT GGA	2261
Leu Val Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala Asn Gly	
425 430 435	
TTA AAA AAT ATC CAC CTT TTC TCA TGG CCC GAG CAC TGT AAA ACT	2306
Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His Cys Lys Thr	
440 445 450	
TAT CTA TCC CGG ATA GCT AGC TGC AAA CCA AGG CAA CCA CGC TGG	2351
Tyr Leu Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln Pro Arg Trp	
455 460 465	
CTG AGA TCC ATT GAT GAT GAT GAT GAA AAT TCA GAA ACA GAT TCA	2396
Leu Arg Ser Ile Asp Asp Asp Asp Glu Asn Ser Glu Thr Asp Ser	
470 475 480	
CCT AGT GAT TCC TTG AGA GAT ATT CAT GAT ATA TCT CTG AAT TTG	2441
Pro Ser Asp Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu	
485 490 495	

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AGA TTT TCA TTA GAT GGG GAA AAG AAT GAC AAT AAA GAA AAT GCT	2486
Arg Phe Ser Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala	
500 505 510	
GAT AAT ACA TTA GAC CCC GAA GTT CGA AGG AGC AAG TTA GAG AAT	2531
Asp Asn Thr Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn	
515 520 525	
GCT GTT TTG TCC TTA TCT AAG GGT GCA CTG AAG AGC ACA TCA AAA	2576
Ala Val Leu Ser Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys	
530 535 540	
TCT TGG TCG TCA GAC AAG GCA GAC CAA AAC CCT GGT GCT GGT AAA	2621
Ser Trp Ser Ser Asp Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys	
545 550 555	
TTC CCA GCG ATT AGG AGG AGG CGA CAT ATT TTT GTT ATT GCA GTG	2666
Phe Pro Ala Ile Arg Arg Arg Arg His Ile Phe Val Ile Ala Val	
560 560 565	
GAT TGT GAT GCT AGC TCA GGA CTC TCT GGA AGT GTG AAA AAG ATA	2711
Asp Cys Asp Ala Ser Ser Gly Leu Ser Gly Ser Val Lys Lys Ile	
570 575 580	
TTT GAG GCT GTA GAG AAG GAA AGG GCA GAG GGT TCC ATT GGA TTT	2756
Phe Glu Ala Val Glu Lys Glu Arg Ala Glu Gly Ser Ile Gly Phe	
585 590 595	
ATC CTG GCT ACA TCT TTC AAT ATA TCA GAA GTA CAG TCT TTC CTG	2801
Ile Leu Ala Thr Ser Phe Asn Ile Ser Glu Val Gln Ser Phe Leu	
600 605 610	
CTT TCA GAG GGC ATG AAT CCT ACT GAT TTT GAT GCT TAC ATA TGC	2846
Leu Ser Glu Gly Met Asn Pro Thr Asp Phe Asp Ala Tyr Ile Cys	
615 620 625	

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AAT AGT GGT GGT GAT CTT TAT TAT TCG TCC TTC CAT TCT GAG CAA	2891
Asn Ser Gly Gly Asp Leu Tyr Tyr Ser Ser Phe His Ser Glu Gln	
630 635 640	
 AAT CCT TTT GTA GTT GAC TTG TAC TAT CAC TCA CAT ATT GAG TAT	2936
Asn Pro Phe Val Val Asp Leu Tyr Tyr His Ser His Ile Glu Tyr	
645 650 655	
 CGT TGG GGG GGC GAA GGA TTG AGA AAG ACT TTG GTG CGT TGG GCC	2981
Arg Trp Gly Gly Glu Gly Leu Arg Lys Thr Leu Val Arg Trp Ala	
660 665 670	
 GCC TCT ATC ATT GAT AAG AAT GGT GAA AAT GGA GAT CAC ATT GTT	3026
Ala Ser Ile Ile Asp Lys Asn Gly Glu Asn Gly Asp His Ile Val	
675 680 685	
 GTT GAG GAT GAA GAC AAT TCA GCT GAC TAC TGC TAT ACT TTC AAA	3071
Val Glu Asp Glu Asp Asn Ser Ala Asp Tyr Cys Tyr Thr Phe Lys	
690 695 700	
 GTC TGC AAG CCT GGG ACG GTT CCT CCA TCT AAA GAG CTT AGA AAA	3116
Val Cys Lys Pro Gly Thr Val Pro Pro Ser Lys Glu Leu Arg Lys	
705 710 715	
 GTA ATG CGA ATT CAG GCA CTT CGT TGT CAC GCT GTT TAT TGT CAA	3161
Val Met Arg Ile Gln Ala Leu Arg Cys His Ala Val Tyr Cys Gln	
720 725 730	
 AAT GGG AGT AGG ATT AAT GTG ATC CCT GTA CTG GCA TCT CGG TCC	3205
Asn Gly Ser Arg Ile Asn Val Ile Pro Val Leu Ala Ser Arg Ser	
735 740 745	
 CAA GCA CTC AGG TAC TTA TAT CTG CGA TGG GGA ATG GAC TTG TCG	3251
Gln Ala Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp Leu Ser	
750 755 760	

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AAG TTG GTG GTT TTC GTC GGA GAA AGT GGT GAT ACC GAT TAT GAA 3296
 Lys Leu Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu
 765 770 775

GGA TTA ATC GGT GGT CTA CGC AAG GCT GTC ATA ATG AAA GGC CTC 3341
 Gly Leu Ile Gly Gly Leu Arg Lys Ala Val Ile Met Lys Gly Leu
 780 785 790

TGC ACT AAT GCA AGC AGC TTA ATT CAC GGT AAT AGG-AAT TAC CCG 3386
 Cys Thr Asn Ala Ser Ser Leu Ile His Gly Asn Arg Asn Tyr Pro
 795 800 805

CTA TCT GAT GTT TTA CCA TTC GAC AGC CCT AAT GTC ATC CAA GCG 3431
 Leu Ser Asp Val Leu Pro Phe Asp Ser Pro Asn Val Ile Gln Ala
 810 815 820

GAC GAG GAA TGT AGC AGC ACC GAA ATC CGT TGC TTA CTG GTG AAA 3476
 Asp Glu Glu Cys Ser Ser Thr Glu Ile Arg Cys Leu Leu Val Lys
 825 830 835

CTA GCG GTA CTC AAA GGA TAATACCCTT CCCCCTTTGA TTGTCAAAAA 3524
 Leu Ala Val Leu Lys Gly
 840

CCTATATGAG CTATAAGACT ATGCCATGAA AAGAATGGCC ATCCATTGCG CTTGTCTTTT 3584

GAAGCTGTTA ATACTTTTCA ACAGACTACA AAATGAGATG AGTCCTTTGA TCCTCTTTAA 3644

AGGACATAAA AGCTTTATGC AAGAACCAGT GCTGTAAAGT TATAGAATTT CTTTGTCTAT 3704

ATATGACATT CGACAGAACC TGTTCCGGTT CATCGA 3740

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52

2. DNA sequence with the coding region for sucrose-phosphate-synthase (SPS) from *Solanum tuberosum* for the preparation of plants with modified sucrose concentration, characterised in that this sequence has the following nucleotide sequence (Seq. ID No.2):

ATTTTTTTCT CTAAGTTCTC TCTCGCTGTC CTTATCATTT CACCACCTCC ATAAATCTAG 60

AAACATCTTT TCTACTCCGT TAATCTCTCT AGCACACGGC GGAGGAGTGC GGCGGAGGAG 120

ATG GCG GGA AAC GAT TGG ATT AAC AGT TAC TTA GAG GCG ATA CTG 165
Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu
1 5 10 15

GAT GTT GGA CCA GGG CTA GAT GAT AAG AAG TCA TCG TTG TTG TTG 210
Asp Val Gly Pro Gly Leu Asp Asp Lys Lys Ser Ser Leu Leu Leu
20 25 30

AGA GAA AGA GGG AGG TTT AGT CCG ACG AGG TAC TTT GTT GAG GAA 255
Arg Glu Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu
35 40 45

GTT ATT ACT GGA TTC GAT GAG ACT GAT TTG CAT CGT TCG TGG ATC 300
Val Ile Thr Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Ile
50 55 60

CGA GCA CAA GCT ACT CGG AGT CCG CAG AGA AGG AAT ACT AGG CTC 345
Arg Ala Gln Ala Thr Arg Ser Pro Gln Arg Arg Asn Thr Arg Leu
65 70 75

GAG AAT ATG TGC TGG AGG ATT TGG AAT TTG GCT CGC CAG AAA AAG 390
Glu Asn Met Cys Trp Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys
80 85 90

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CAG CTT GAG GGA GAG CAA GCT CAG TGG ATG GCA AAA CGC CGT CAA	435
Gln Leu Glu Gly Glu Gln Ala Gln Trp Met Ala Lys Arg Arg Gln	
95 100 105	
GAA CGT GAA AGA GGT CGC AGA GAA GCA GTT GCT GAT ATG TCA GAG	480
Glu Arg Glu Arg Gly Arg Arg Glu Ala Val Ala Asp Met Ser Glu	
110 115 120	
GAT CTA TCT GAG GGA GAG AAA GGA GAT ATA GTC GCT GAC ATG TCA	525
Asp Leu Ser Glu Gly Glu Lys Gly Asp Ile Val Ala Asp Met Ser	
125 130 135	
TCT CAT GGT GAA AGT ACC AGA GGC CGA TTG CCT AGA ATC AGT TCT	570
Ser His Gly Glu Ser Thr Arg Gly Arg Leu Pro Arg Ile Ser Ser	
140 145 150	
GTT GAG ACA ATG GAA GCA TGG GTC AGT CAG CAG AGA GGA AAG AAG	615
Val Glu Thr Met Glu Ala Trp Val Ser Gln Gln Arg Gly Lys Lys	
155 160 165	
CTT TAT ATC GTG CTT ATA AGT TTA CAT GGT TTA ATT CGG GGT GAG	660
Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu Ile Arg Gly Glu	
170 175 180	
AAT ATG GAG CTT GGA CGG GAT TCT GAT ACT GGT GGT CAG GTG AAG	705
Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys	
185 190 195	
TAT GTT GTT GAA CTT GCG AGG GCC TTA GGG TCG ATG CCA GGT GTA	750
Tyr Val Val Glu Leu Ala Arg Ala Leu Gly Ser Met Pro Gly Val	
200 205 210	
TAT CGG GTT GAC TTG CTT ACT AGA CAA GTA TCT TCA CCA GAA GTA	795
Tyr Arg Val Asp Leu Leu Thr Arg Gln Val Ser Ser Pro Glu Val	
215 220 225	

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GAT TGG AGC TAT GGT GAG CCG ACA GAG ATG CTG ACG CCA ATA AGT	840
Asp Trp Ser Tyr Gly Glu Pro Thr Glu Met Leu Thr Pro Ile Ser	
230 235 240	
ACA GAC GGC TTG ATG ACT GAG ATG GGG GAG AGT AGT GGT GCT TAT	885
Thr Asp Gly Leu Met Thr Glu Met Gly Glu Ser Ser Gly Ala Tyr	
245 250 255	
ATT ATT CGC ATT CCT TTT GGA CCA AGA GAG AAA TAT ATT CCA AAA	930
Ile Ile Arg Ile Pro Phe Gly Pro Arg Glu Lys Tyr Ile Pro Lys	
260 265 270	
GAA CAG CTA TGG CCC TAT ATT CCC GAA TTT GTT GAT GGT GCA CTT	975
Glu Gln Leu Trp Pro Tyr Ile Pro Glu Phe Val Asp Gly Ala Leu	
275 280 285	
AAC CAT ATT ATT CAA ATG TCC AAA GTT CTT GGG GAG CAA ATT GGT	1020
Asn His Ile Ile Gln Met Ser Lys Val Leu Gly Glu Gln Ile Gly	
290 295 300	
AGT GGC TAT CCT GTG TGG CCT GTT GCC ATA CAC GGA CAT TAT GCT	1065
Ser Gly Tyr Pro Val Trp Pro Val Ala Ile His Gly His Tyr Ala	
305 310 315	
GAT GCT GGC GAC TCA GCT GCT CTC CTG TCA GGT GCT TTA AAT GTA	1110
Asp Ala Gly Asp Ser Ala Ala Leu Leu Ser Gly Ala Leu Asn Val	
320 330 335	
CCA ATG CTT TTC ACT GGT CAC TCA CTT GGT AGA GAT AAG TTG GAG	1155
Pro Met Leu Phe Thr Gly His Ser Leu Gly Arg Asp Lys Leu Glu	
340 345 350	
CAA CTG TTG GCA CAA GGT CGA AAG TCA AAG GAT GAA ATA AAC TCA	1200
Gln Leu Leu Ala Gln Gly Arg Lys Ser Lys Asp Glu Ile Asn Ser	
355 360 365	

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ACC TAC AAG ATA ATG CGG AGA ATA GAG GCT GAA GAA TTA ACT CTT	1245
Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu Glu Leu Thr Leu	
370 375 380	
GAT GCT TCC GAA ATT GTC ATC ACT AGT ACA AGA CAG GAG ATT GAC	1290
Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg Gln Glu Ile Asp	
385 390 395	
GAG CAA TGG CGT TTG TAT GAT GGG TTT GAT CCA ATA TTA GAG CGT	1335
Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro Ile Leu Glu Arg	
400 405 410	
AAG TTA CGT GCA AGG ATC AAG CGC AAT GTC AGC TGT TAT GGC AGG	1380
Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser Cys Tyr Gly Arg	
415 420 425	
TTT ATG CCT CGT ATG GCT GTA ATT CCT CCT GGG ATG GAG TTC CAC	1425
Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly Met Glu Phe His	
430 435 440	
CAT ATT GTG CCA CAT GAA GGT GAC ATG GAT GGT GAA ACA GAA GGA	1470
His Ile Val Pro His Glu Gly Asp Met Asp Gly Glu Thr Glu Gly	
445 450 455	
AGT GAA GAT GGG AAG ACC CCG GAT CCA CCT ATT TGG GCA GAG ATT	1515
Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile Trp Ala Glu Ile	
460 465 470	
ATG CGC TTC TTT TCT AAT CCA AGG AAG CCT ATG ATA CTC GCA CTT	1560
Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met Ile Leu Ala Leu	
475 480 485	
GCT AGG CCT GAT CCC AAG AAG AAC CTC ACT ACT TTA GTG AAA GCA	1605
Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr Leu Val Lys Ala	
490 495 500	

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TTT GGT GAA TGT CGT CCA TTG AGA GAG CTT GCT AAT CTT ACT TTG	1650
Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu	
505 510 515	
ATA ATG GGT AAT CGA GAT AAT ATC GAC GAA ATG TCT AGC ACC AAT	1695
Ile Met Gly Asn Arg Asp Asn Ile Asp Glu Met Ser Ser Thr Asn	
520 525 530	
TCT GCA CTT CTT CTT TCA ATC TTG AAA ATG ATA GAT AAG TAT GAT	1740
Ser Ala Leu Leu Leu Ser Ile Leu Lys Met Ile Asp Lys Tyr Asp	
535 540 540	
CTT TAT GGT CAA GTA GCT TAT CCT AAA CAC CAC AAG CAG TCA GAT	1785
Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys Gln Ser Asp	
545 550 555	
GTT CCT GAT ATC TAC CGT CTT GCT GCA AAG ACT AAG GGT GTT TTT	1830
Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly Val Phe	
560 565 570	
ATT AAT CCA GCT TTT ATT GAG CCT TTT GGA CTG ACT TTG ATT GAG	1875
Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu Thr Leu Ile Glu	
575 580 585	
GCA GCA GCT TAT GGT CTC CCA ATG GTA GCC ACA AAA AAT GGA GGA	1920
Ala Ala Ala Tyr Gly Leu Pro Met Val Ala Thr Lys Asn Gly Gly	
590 595 600	
CCT GTT GAT ATA CAT AGG GTT CTT GAC AAT GGT CTC TTA GTG GAT	1965
Pro Val Asp Ile His Arg Val Leu Asp Asn Gly Leu Leu Val Asp	
605 610 615	
CCC CAT GAT CAG CAG GCA ATT GCT GAT GCT CTT TTG AAG TTG GTT	2010
Pro His Asp Gln Gln Ala Ile Ala Asp Ala Leu Leu Lys Leu Val	
620 625 630	

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GCT GAT AAG CAA CTG TGG GCT AAA TGC AGG GCA AAT GGA TTA AAA	2055
Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala Asn Gly Leu Lys	
635 640 645	
AAT ATC CAC CTT TTC TCA TGG CCC GAG CAC TGT AAA ACT TAT CTA	2100
Asn Ile His Leu Phe Ser Trp Pro Glu His Cys Lys Thr Tyr Leu	
650 655 660	
TCC CGG ATA GCT AGC TGC AAA CCA AGG CAA CCA CGC TGG CTG AGA	2145
Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln Pro Arg Trp Leu Arg	
665 670 675	
TCC ATT GAT GAT GAT GAT GAA AAT TCA GAA ACA GAT TCA CCT AGT	2190
Ser Ile Asp Asp Asp Asp Glu Asn Ser Glu Thr Asp Ser Pro Ser	
680 685 690	
GAT TCC TTG AGA GAT ATT CAT GAT ATA TCT CTG AAT TTG AGA TTT	2235
Asp Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu Arg Phe	
695 700 705	
TCA TTA GAT GGG GAA AAG AAT GAC AAT AAA GAA AAT GCT GAT AAT	2280
Ser Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala Asp Asn	
710 715 720	
ACA TTA GAC CCC GAA GTT CGA AGG AGC AAG TTA GAG AAT GCT GTT	2325
Thr Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn Ala Val	
725 730 735	
TTG TCC TTA TCT AAG GGT GCA CTG AAG AGC ACA TCA AAA TCT TGG	2370
Leu Ser Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys Ser Trp	
740 745 750	
TCG TCA GAC AAG GCA GAC CAA AAC CCT GGT GCT GGT AAA TTC CCA	2415
Ser Ser Asp Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys Phe Pro	
755 760 765	

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GCG ATT AGG AGG AGG CGA CAT ATT TTT GTT ATT GCA GTG GAT TGT	2460
Ala Ile Arg Arg Arg Arg His Ile Phe Val Ile Ala Val Asp Cys	
770 775 780	
GAT GCT AGC TCA GGA CTC TCT GGA AGT GTG AAA AAG ATA TTT GAG	2505
Asp Ala Ser Ser Gly Leu Ser Gly Ser Val Lys Lys Ile Phe Glu	
785 790 795	
GCT GTA GAG AAG GAA AGG GCA GAG GGT TCC ATT GGA TTT ATC CTG	2550
Ala Val Glu Lys Glu Arg Ala Glu Gly Ser Ile Gly Phe Ile Leu	
800 805 810	
GCT ACA TCT TTC AAT ATA TCA GAA GTA CAG TCT TTC CTG CTT TCA	2595
Ala Thr Ser Phe Asn Ile Ser Glu Val Gln Ser Phe Leu Leu Ser	
815 820 825	
GAG GGC ATG AAT CCT ACT GAT TTT GAT GCT TAC ATA TGC AAT AGT	2640
Glu Gly Met Asn Pro Thr Asp Phe Asp Ala Tyr Ile Cys Asn Ser	
830 835 840	
GGT GGT GAT CTT TAT TAT TCG TCC TTC CAT TCT GAG CAA AAT CCT	2685
Gly Gly Asp Leu Tyr Tyr Ser Ser Phe His Ser Glu Gln Asn Pro	
845 850 855	
TTT GTA GTT GAC TTG TAC TAT CAC TCA CAT ATT GAG TAT CGT TGG	2730
Phe Val Val Asp Leu Tyr Tyr His Ser His Ile Glu Tyr Arg Trp	
860 865 870	
GGG GGC GAA GGA TTG AGA AAG ACT TTG GTG CGT TGG GCC GCC TCT	2775
Gly Gly Glu Gly Leu Arg Lys Thr Leu Val Arg Trp Ala Ala Ser	
875 880 885	
ATC ATT GAT AAG AAT GGT GAA AAT GGA GAT CAC ATT GTT GTT GAG	2820
Ile Ile Asp Lys Asn Gly Glu Asn Gly Asp His Ile Val Val Glu	
890 895 900	

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GAT GAA GAC AAT TCA GCT GAC TAC TGC TAT ACT TTC AAA GTC TGC	2865
Asp Glu Asp Asn Ser Ala Asp Tyr Cys Tyr Thr Phe Lys Val Cys	
905 910 915	
AAG CCT GGG ACG GTT CCT CCA TCT AAA GAG CTT AGA AAA GTA ATG	2910
Lys Pro Gly Thr Val Pro Pro Ser Lys Glu Leu Arg Lys Val Met	
920 925 930	
CGA ATT CAG GCA CTT CGT TGT CAC GCT GTT TAT TGT CAA AAT GGG	2955
Arg Ile Gln Ala Leu Arg Cys His Ala Val Tyr Cys Gln Asn Gly	
935 940 945	
AGT AGG ATT AAT GTG ATC CCT GTA CTG GCA TCT CGG TCC CAA GCA	3000
Ser Arg Ile Asn Val Ile Pro Val Leu Ala Ser Arg Ser Gln Ala	
950 955 960	
CTC AGG TAC TTA TAT CTG CGA TGG GGA ATG GAC TTG TCG AAG TTG	3045
Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp Leu Ser Lys Leu	
965 970 975	
GTG GTT TTC GTC GGA GAA AGT GGT GAT ACC GAT TAT GAA GGA TTA	3090
Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu Gly Leu	
980 985 990	
ATC GGT GGT CTA CGC AAG GCT GTC ATA ATG AAA GGC CTC TGC ACT	3135
Ile Gly Gly Leu Arg Lys Ala Val Ile Met Lys Gly Leu Cys Thr	
995 1000 1005	
AAT GCA AGC AGC TTA ATT CAC GGT AAT AGG AAT TAC CCG CTA TCT	3180
Asn Ala Ser Ser Leu Ile His Gly Asn Arg Asn Tyr Pro Leu Ser	
1010 1015 1020	
GAT GTT TTA CCA TTC GAC AGC CCT AAT GTC ATC CAA GCG GAC GAG	3225
Asp Val Leu Pro Phe Asp Ser Pro Asn Val Ile Gln Ala Asp Glu	
1025 1030 1035	

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GAA TGT AGC AGC ACC GAA ATC CGT TGC TTA CTG GAG AAA CTA GCG 3270
 Glu Cys Ser Ser Thr Glu Ile Arg Cys Leu Leu Glu Lys Leu Ala
 1040 1045 1050

GTA CTC AAA GGA TAA TACCCTTCCC CCTTTGATTG TCAAAAACCT 3315
 -Val Leu Lys Gly End
 1054

ATATGAGCTA TAAGACTATG CCATGAAAAG AATGGCCATC CATTTGGCTT GTCTTTTGAA 3375
 CCTGTTAATA CTTTCAACA GACTACAAA TGAGATGAGT CCTTTGATCC TCTTTAAAG 3435
 ACATAAAGC TTTATGCAAG AACCAAGTGT GTAAAGTTAT AGAATTTCTT TTGCTATATA 3495
 TGACATTCGA CAGAACCAGT TCCGGTTCAT CGAGAAAAAG AAATAAATTT CAACTTATAA 3555
 ACATGCCTGA TCATGTAAAT TATCATATAC ATCCATCGGA AGGCATTATC GATGGGTTAT 3615
 CAGATTTTTT 3625

3. DNA sequence with the coding region for
 sucrose-phosphate-synthase (SPS) from *Solanum*
tuberosum for the preparation of plants with
 modified sucrose concentration, characterised in
 5 that this sequence has the following nucleotide
 sequence (Seq. ID No.3):

ATTTTTT TCTCTAAATT CTCTCTCACT GTCCTTATCA TTTCACCACC TCCATAAATC 57

TAGAAACATC TTTTCTATTC CGTTAATCTC TCTAGCACAC GGCGGAGTGC GGCGGAGGAG 117

ATG GCG GGA AAC GAC TGG ATT AAC AGT TAC TTA GAG GCG ATA CTG 162
 Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu
 1 5 10 15

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GAT GTA GGA CCA GGG CTA GAT GAT AAG AAA TCA TCG TTG TTG TTG	207
Asp Val Gly Pro Gly Leu Asp Asp Lys Lys Ser Ser Leu Leu Leu	
20 25 30	
AGA GAA AGA GGG AGG TTT AGT CCG ACG AGG TAC TTT GTT GAG GAA	252
Arg Glu Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu	
35 40 45	
GTT ATT ACT GGA TTC GAT GAG ACT GAT TTG CAT CGC TCG TGG ATC	297
Val Ile Thr Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Ile	
50 55 60	
CGA GCA CAA GCT ACT CGG AGT CCG CAG GAG AGG AAT ACT AGG CTC	342
Arg Ala Gln Ala Thr Arg Ser Pro Gln Glu Arg Asn Thr Arg Leu	
65 70 75	
GAG AAT ATG TGC TGG AGG ATT TGG AAT TTG GCT CGC CAG AAA AAG	387
Glu Asn Met Cys Trp Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys	
80 85 90	
CAG CTT GAG GGA GAG CAA GCT CAG TGG ATG GCA AAA CGC CGT CAA	432
Gln Leu Glu Gly Glu Gln Ala Gln Trp Met Ala Lys Arg Arg Gln	
95 100 105	
GAA CGT GAG AGA GGT CGC AGA GAA GCA GTT GCT GAT ATG TCA GAG	477
Glu Arg Glu Arg Gly Arg Arg Glu Ala Val Ala Asp Met Ser Glu	
110 115 120	
GAT CTA TCT GAG GGA GAG AAA GGA GAT ATA GTC GCT GAC ATG TCA	522
Asp Leu Ser Glu Gly Glu Lys Gly Asp Ile Val Ala Asp Met Ser	
125 130 135	
TCT CAT GGT GAA AGT ACC AGA GGC CGA TTG CCT AGA ATC AGT TCT	567
Ser His Gly Glu Ser Thr Arg Gly Arg Leu Pro Arg Ile Ser Ser	
140 145 150	

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GTT GAG ACA ATG GAA GCA TGG GTC AGT CAG CAG AGA GGA AAG AAG	612
Val Glu Thr Met Glu Ala Trp Val Ser Gln Gln Arg Gly Lys Lys	
155 160 165	
CTT TAT ATC GTG CTT ATA AGT TTA CAT GGT TTA ATT CGG GGT GAG	657
Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu Ile Arg Gly Glu	
170 175 180	
AAT ATG GAG CTT GGA CGG GAT TCT GAT ACT GGT GGT CAG GTG AAG	702
Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys	
185 190 195	
TAT GTA GTT GGA GCA ACT GTT GCA CAA GGT CGT TTG TCA AAG GAT	747
Tyr Val Val Gly Ala Thr Val Ala Gln Gly Arg Leu Ser Lys Asp	
200 205 210	
GAA ATA AAC TCA ACC TAC AAG ATA ATG CGG AGA ATA GAG GCT GAA	792
Glu Ile Asn Ser Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu	
215 220 225	
GAA TTA ACT CTT GAT GCT TCC GAA ATT GTC ATC ACT AGT ACA AGA	837
Glu Leu Thr Leu Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg	
230 235 240	
CAG GAG ATT GAC GAG CAA TGG CGT TTG TAT GAT GGG TTT GAT CCA	882
Gln Glu Ile Asp Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro	
245 250 255	
ATA TTA GAG CGT AAG TTA CGT GCA AGG ATC AAG CGC AAT GTC AGC	927
Ile Leu Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser	
260 265 270	
TGT TAT GGC AGG TTT ATG CCT CGT ATG GCT GTA ATT CCT CCT GGG	972
Cys Tyr Gly Arg Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly	
275 280 285	

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ATG GAG TTC CAC CAT ATT GTG CCA CAT GAA GGT GAC ATG GAT GGT	1017
Met Glu Phe His His Ile Val Pro His Glu Gly Asp Met Asp Gly	
290 295 300	
GAA ACA GAA GGA AGT GAA GAT GGA AAG ACC CCG GAT CCA CCT ATT	1062
Glu Thr Glu Gly Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile	
305 310 315	
TGG GCA GAG ATT ATG CGC TTC TTT TCT AAT CCA AGG AAG CCT ATG	1107
Trp Ala Glu Ile Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met	
320 330 335	
ATA CTC GCA CTT GCT AGG CCT GAT CCC AAG AAG AAC CTC ACT ACT	1152
Ile Leu Ala Leu Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr	
340 345 350	
TTA GTG AAA GCA TTT GGT GAA TGT CGT CCA TTG AGA GAC CTT GCT	1197
Leu Val Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Asp Leu Ala	
355 360 365	
AAT CTT ACT TTG ATA ATG GGT AAT CGA GAT AAT ATC GAC GAA ATG	1242
Asn Leu Thr Leu Ile Met Gly Asn Arg Asp Asn Ile Asp Glu Met	
370 375 380	
TCT AGC ACC AAT TCT GCA CTT CTT CTT TCA ATC TTG AAG ATG ATA	1287
Ser Ser Thr Asn Ser Ala Leu Leu Leu Ser Ile Leu Lys Met Ile	
385 390 395	
GAT AAG TAT GAT CTT TAT GGT CTA GTA GCT TAT CCT AAA CAC CAC	1332
Asp Lys Tyr Asp Leu Tyr Gly Leu Val Ala Tyr Pro Lys His His	
400 405 410	
AAG CAG TCA GAT GTT CCT GAT ATC TAC CGT CTT GCT GCA AAG ACT	1377
Lys Gln Ser Asp Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr	
415 420 425	

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AAG GGT GTT TTT ATT AAT CCA GCT TTT ATT GAG CCT TTT GGA CTG	1422
Lys Gly Val Phe Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu	
430 435 440	
ACT TTG ATT GAG GCA GCA GCT TAT GGT CTC CCA ATG GTA GCC ACA	1467
Thr Leu Ile Glu Ala Ala Ala Tyr Gly Leu Pro Met Val Ala Thr	
445 450 455	
AAA AAT GGA GGA CCT GTT GAT ATA CAT AGG GTT CTT GAC AAT GGT	1512
Lys Asn Gly Gly Pro Val Asp Ile His Arg Val Leu Asp Asn Gly	
460 465 470	
CTC TTA GTG GAT CCC CAT GAT CAG CAG GCA ATT GCT GAT GCT CTT	1557
Leu Leu Val Asp Pro His Asp Gln Gln Ala Ile Ala Asp Ala Leu	
475 480 485	
TTG AAG TTG GTT GCT GAT AAG CAA CTG TGG GCT AAA TGC AGG GCA	1602
Leu Lys Leu Val Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala	
490 495 500	
AAT GGA TTA AAA AAT ATC CAC CTT TTC TCA TGG CCC GAG CAC TGT	1647
Asn Gly Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His Cys	
505 510 515	
AAA ACT TAT CTA TCC CGG ATA GCT AGC TGC AAA CCG AGG CAA CAT	1692
Lys Thr Tyr Leu Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln His	
520 525 530	
TCC TTG AGA GAT ATT CAT GAT ATA TCT CTG AAT TTG AGA TTT TCA	1737
Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu Arg Phe Ser	
535 540 540	
TTA GAT GGG GAA AAG AAT GAC AAT AAA GAA AAT GCT GAT AAT ACA	1782
Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala Asp Asn Thr	
545 550 555	

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65

TTA GAC CCC GAA GTT CGA AGG AGC AAG TTA GAG AAT GCT GTT TTG	1827
Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn Ala Val Leu	
560 565 570	
TCC TTA TCT AAG GGT GCA CTG AAG AGC ACA TCA AAA TCT TGG TCG	1872
Ser Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys Ser Trp Ser	
575 580 585	
TCA GAC AAG GCA GAC CAA AAT CCT GGT GCT GGT AAA TTC CCA GCG	1917
Ser Asp Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys Phe Pro Ala	
590 595 600	
ATT AGG AGG AGG CGA CAT ATT TTT GTT ATT GCA GTG GAT TGT GAT	1962
Ile Arg Arg Arg Arg His Ile Phe Val Ile Ala Val Asp Cys Asp	
605 610 615	
GCT AGC TCA GGA CTC TCT GGA AGT ATG AAA AAG ATA TTT GAG GCT	2007
Ala Ser Ser Gly Leu Ser Gly Ser Met Lys Lys Ile Phe Glu Ala	
620 625 630	
GTA GAG AAG GAA AGG GCA GAG GGT TCC ATT GGA TTT ATC CTT GCT	2052
Val Glu Lys Glu Arg Ala Glu Gly Ser Ile Gly Phe Ile Leu Ala	
635 640 645	
ACA TCT TTC AAT ATA TCA GAA GTA CAG TCT TTC CTG CTT TCA GAG	2097
Thr Ser Phe Asn Ile Ser Glu Val Gln Ser Phe Leu Leu Ser Glu	
650 655 660	
GGC ATG AAT CCT ACT GAG CAA AAT CCT TTT GTA GTT GAC TTG TAC	2142
Gly Met Asn Pro Thr Glu Gln Asn Pro Phe Val Val Asp Leu Tyr	
665 670 675	
TAT CAC TCA CAT ATT GAG TAT CGT TGG GGG GGC GAA GGG TTG AGA	2187
Tyr His Ser His Ile Glu Tyr Arg Trp Gly Gly Glu Gly Leu Arg	
680 685 690	

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AAG ACT TTG GTG CGT TGG GCC GCC TCT ATC ATT GAT AAG AAT GGT	2232
Lys Thr Leu Val Arg Trp Ala Ala Ser Ile Ile Asp Lys Asn Gly	
695 700 705	
GAA AAT GGA GAT CAC ATT GTT GTT GAG GAT GAA GAC AAT TCA GCT	2277
Glu Asn Gly Asp His Ile Val Val Glu Asp Glu Asp Asn Ser Ala	
710 715 720	
GAC TAC TGC TAT ACA TTC AAA GTT TGC AAG CCT GGG ACG GTT CCT	2322
Asp Tyr Cys Tyr Thr Phe Lys Val Cys Lys Pro Gly Thr Val Pro	
725 730 735	
CCA TCT AAA GAA CTT AGA AAA GTA ATG CGA ATT CAG GCA CTT CGT	2367
Pro Ser Lys Glu Leu Arg Lys Val Met Arg Ile Gln Ala Leu Arg	
740 745 750	
TGT CAC GCT GTT TAT TGT CAA AAT GGG AGT AGG ATT AAT GTG ATC	2412
Cys His Ala Val Tyr Cys Gln Asn Gly Ser Arg Ile Asn Val Ile	
755 760 765	
CCT GTA CTG GCA TCT CGG TCC CAA GCA CTC AGG TAC TTA TAT CTG	2457
Pro Val Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Leu	
770 775 780	
CGA TGG GGA ATG GTC CCT GTA CTG GCA TCT CGG TCC CAA GCA CTC	2502
Arg Trp Gly Met Val Pro Val Leu Ala Ser Arg Ser Gln Ala Leu	
785 790 795	
AGG TAC TTA TAT CTG CGA TGG GGA ATG GTC CCT GTA CTG GCA TCT	2547
Arg Tyr Leu Tyr Leu Arg Trp Gly Met Val Pro Val Leu Ala Ser	
800 805 810	
CGG TCC CAA GCA CTC AGG TAC TTA TAT CTG CGA TGG GGA ATG GAC	2592
Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp	
815 820 825	

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TTG TCG AAG TTG GTG GTT TTC GTC GGA GAA AGT GGT GAT ACC GAT 2637
 Leu Ser Lys Leu Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp
 830 835 840

TAT GAA GGA TTG ATC GGT GGT CTA CGC AAG GCT GTC ATA ATG AAA 2682
 Tyr Glu Gly Leu Ile Gly Gly Leu Arg Lys Ala Val Ile Met Lys
 845 850 855

GGA CTC TGC ACT AAT GCA AGC AGC TTA ATT CAC GGT AAT AGG AAT 2727
 Gly Leu Cys Thr Asn Ala Ser Ser Leu Ile His Gly Asn Arg Asn
 860 865 870

TAC CCG CTA TCT GAT GTT TTA CCA TTC GAG AGC CCT AAT GTC ATC 2772
 Tyr Pro Leu Ser Asp Val Leu Pro Phe Glu Ser Pro Asn Val Ile
 875 880 885

CAA GCG GAT GAG GAA TGT AGC AGC ACC GGA ATC CGT TCC TTA CTG 2817
 Gln Ala Asp Glu Glu Cys Ser Ser Thr Gly Ile Arg Ser Leu Leu
 905 910 915

GAG AAA CTA GCG GTA CTC AAA GGA TAA TACCCTTCCC CCTTTGATTG 2864
 Glu Lys Leu Ala Val Leu Lys Gly End
 920

TCAAAAACCT ATATGAGCTA AGATTATGCC ATGAAAAGAA TGGCCATCCA TTTGGCTTGT2924

CTTTTG 2930

4. Derivatives of DNA sequences according to any one of claims 1 to 3, characterised in that these derivatives are obtained by exchange of single bases or by deletion or insertion of base sequences and which code for proteins with a comparable activity to sucrose phosphate synthase.
- 5

SUBSTITUTE SHEET

to 7 for the preparation of plants with changed sucrose concentration.

- 5 10. Use of the plasmid p35S-anti-pot-SPS for the preparation of plants with reduced mRNA concentration for the SPS protein and reduced enzyme activity.
- 10 11. Use of the plasmid p35S-anti-pot-SPS for the preparation of plants with reduced mRNA concentration for the SPS protein and reduced enzyme activity, specifically in the tuber.
- 15 12. Use of the DNA sequences according to any one of claims 1 to 3 for the preparation of derivatives by targeted or non-targeted mutagenesis.
- 20 13. Use of the DNA sequences according to any one of claims 1 to 3 for the preparation of derivatives, whose gene products are not subjected to the plant's own activity regulation during a phosphorylation reaction.
- 25 14. Use of the DNA sequences according to any one of claims 1 to 3 for the preparation of derivatives, whose gene products are not neutralized by the plant's own activity regulation during a phosphorylation reaction.
- 30 15. Transgenic plants, whose sugar metabolism is modified by introduction of one or several of the DNA sequences according to any one of claims 1 to 3.
- 35 16. Plants according to claim 15, characterised in that it is a potato.

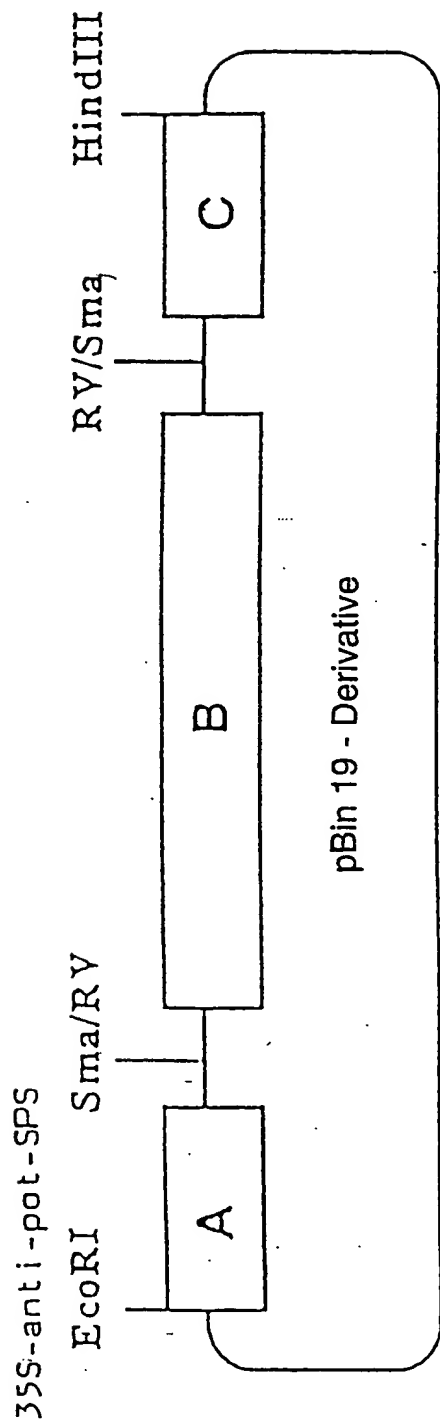


Fig. 1

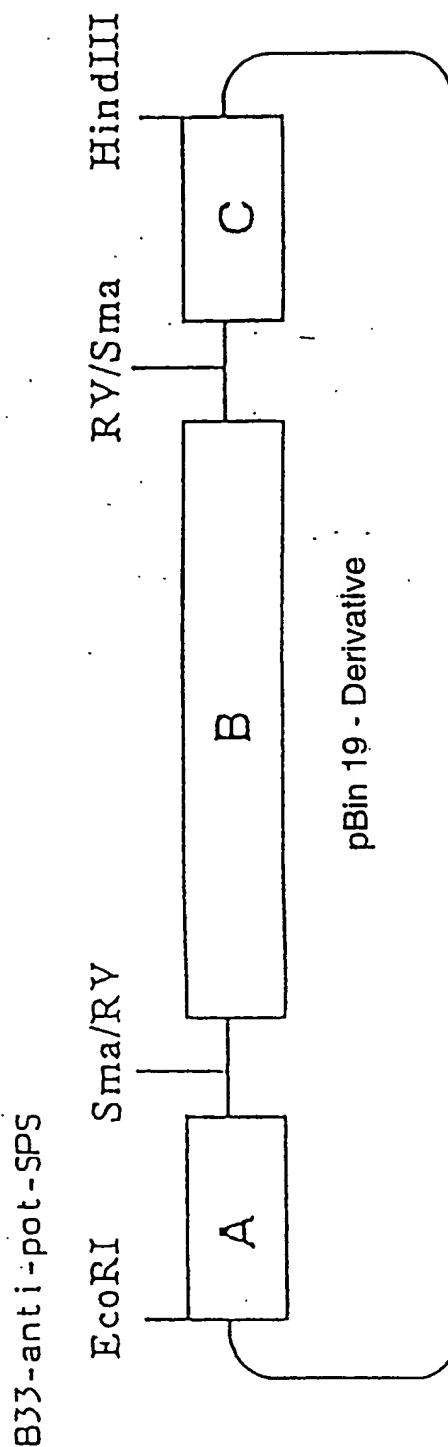


Fig. 2

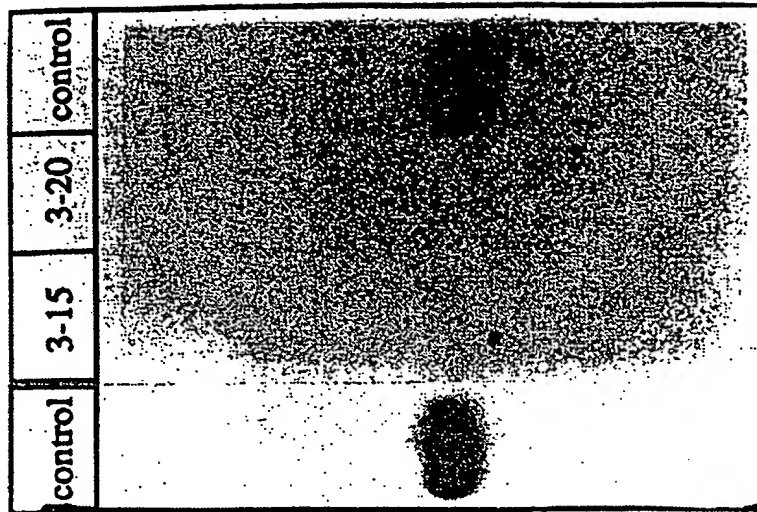


Fig. 4

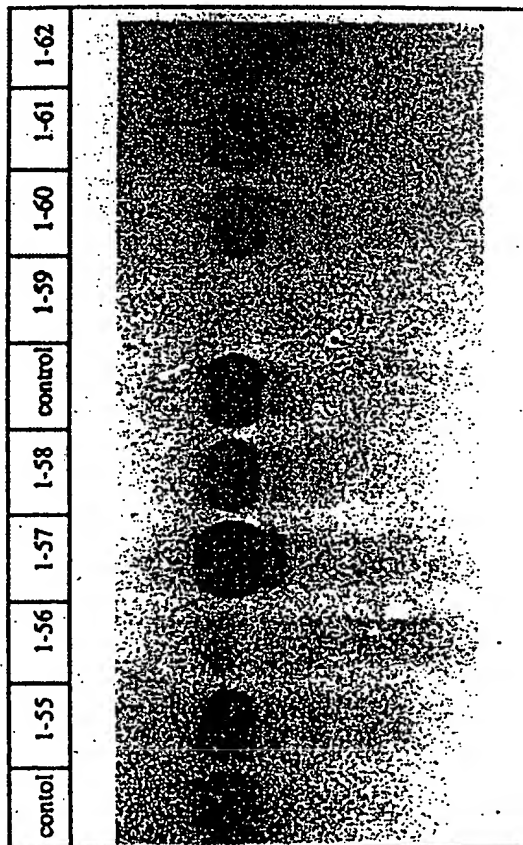


Fig. 3

SUBSTITUTE SHEET

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

PCT/EP 93/01505

REC'D 23 JUL 1993

WIPO PCT

INTERNATIONAL FORM

Institut für Genbiologische
Forschung Berlin GmbH
Innestrasse 63
1000 Berlin 33

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR pB33-anti-pot-sps	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 7124
II. SCIENTIFIC DESCRIPTION AND/OR TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: () a scientific description () a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts this microorganism identified under I. above, which was received by it on 1992-06-12 (Date of original deposit) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Adress: Mascheroder Weg 1 B D-3300 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiks Date: 1992-06-30

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
 RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
 FOR THE PURPOSES OF PATENT PROCEDURE

RECEIVED 25 JUL 1993
 DEPOSITARY AUTHORITY

INTERNATIONAL FORM

Institut für Genbiologische
 Forschung Berlin GmbH
 Ihnestr. 63
 1000 Berlin 33

VIABILITY STATEMENT

issued pursuant to Rule 10.2 by the
 INTERNATIONAL DEPOSITARY AUTHORITY
 identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Institut für Genbiologische Forschung Berlin GmbH Address: Ihnestr. 63 1000 Berlin 33	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 7124 Date of the deposit or of the transfer ¹ : 1992-06-12
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 1992-06-15. ² On that date, the said microorganism was (X) ³ viable () ³ no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ⁴	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSM DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1 B D-3300 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>O. Weh</i> Date: 1992-06-30

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

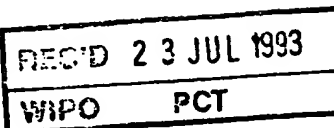
³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.

PCT/EP 93 / 0 16 0 5

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM



Institut für Genbiologische
Forschung Berlin GmbH
Innestrasse 63
1000 Berlin 33

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR p35S-anti-pot-sps	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 7125
II. SCIENTIFIC DESCRIPTION AND/OR TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: () a scientific description () a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts this microorganism identified under I. above, which was received by it on 1992-06-12 (Date of original deposit) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Adress: Mascheroder Weg 1 B D-3300 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>U. Weiler</i> Date: 1992-06-30

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

F.T/EP 93 / 0 1 6 0 5

REC'D 23 JUL 1993

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Institut für Genbiologische
Forschung Berlin GmbH
Innestrasse 63
1000 Berlin 33

VIABILITY STATEMENT

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I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Institut für Genbiologische Forschung Berlin GmbH Address: Innestrasse 63 1000 Berlin 33	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 7125 Date of the deposit or of the transfer ¹ : 1992-06-12
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 1992-06-15 ² On that date, the said microorganism was (X) ³ viable () ³ no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED⁴	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSM DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1 B D-3300 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>U. Weis</i> Date: 1992-06-30

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 5 C12N9/10 C12N15/82 C12N15/11 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT CELL. vol. 3, no. 10, October 1991, ROCKVILLE, MD, USA. pages 1121 - 1130 WORRELL, A.C., ET AL. 'Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning' see the whole document	5,9
Y	---	5,9
X	THE PLANT JOURNAL vol. 1, no. 1, 1991 pages 51 - 58 QUICK, W.P., ET AL. 'The impact of decreased Rubisco on photosynthesis, growth, allocation and storage in tobacco plants which have been transformed with antisense rbcS' see the whole document	5,9

	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *&* document member of the same patent family

Date of the actual completion of the international search

8 November 1993

Date of mailing of the international search report

12 93

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

MADDOX, A

INTERNATIONAL SEARCH REPORT

 Inter. natl Application No
 PCT/EP 93/01605

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PLANT PHYSIOLOGY. vol. 99, no. 1, May 1992, ROCKVILLE, MD, USA. page 12 SONNEWALD, U., ET AL. 'Molecular approaches to influence sink-source interactions in transgenic plants' see abstract 67 ---	5,9
X	EP,A,0 438 904 (ADVANCED TECHNOLOGIES) 31 July 1991 see page 7, line 40 - line 60 ---	5,9
X	EP,A,0 485 044 (IGFB) 13 May 1992 see the whole document ---	5,9
X	EP,A,0 466 995 (ROUSSEL-UCLAF) 22 January 1992 see the whole document ---	5
P,X	WO,A,92 16631 (ROUSSEL-UCLAF) 1 October 1992 see the whole document ---	5,9
P,X	EP,A,0 530 978 (ADVANCED TECHNOLOGIES) 10 March 1993 see the whole document ---	5,9
A	EP,A,0 455 316 (IGFB) 6 November 1991 see the whole document ---	1-16
A	BIOLOGICAL ABSTRACTS vol. 55 1973, Philadelphia, PA, US; abstract no. 68960, MURATA, T. 'Sucrose phosphate synthetase from various plant origins' see abstract & AGRIC. BIOL. CHEM. vol. 36, no. 11, 1972 pages 1877 - 1884 ---	1-16
A	BIOLOGICAL ABSTRACTS vol. 80 1985, Philadelphia, PA, US; abstract no. 85644, SOWOKINOS, J.R., ET AL. 'Translucent tissue defects in Solanum tuberosum: 1. Alterations in amyloplast membrane integrity, enzyme activities, sugars and starch content' see abstract & PLANT PHYSIOL vol. 78, no. 3, 1985 pages 489 - 494 -----	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 93/01605

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0438904	31-07-91	AU-A- 6836590 JP-A- 4341126	04-07-91 27-11-92
EP-A-0485044	13-05-92	DE-A- 4035756 AU-A- 8702191 CA-A- 2055150	14-05-92 14-05-92 09-05-92
EP-A-0466995	22-01-92	AU-A- 8394591 WO-A- 9201782 JP-T- 5502169	18-02-92 06-02-92 22-04-93
WO-A-9216631	01-10-92	AU-A- 8765591 EP-A- 0533849	21-10-92 31-03-93
EP-A-0530978	10-03-93	NONE	
EP-A-0455316	06-11-91	DE-A- 4013144	24-10-91

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